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ERRATA

Page 71, *read* Fig. 29 *for* left-hand figure, and Fig. 28 *for* right-hand figure.

Page 127, title, *read* 27 to December 30, 1927.

Page 141, the title to *read* A preliminary report on the relationship of insect barriers to the development of tip and margin burn of Irish potatoes *for* A preliminary report on the relationship of insect carriers, etc.

Page 215, line 8, *read* corms *for* corns.

Page 274, Table 7, the 2-column heading, *read* No manure | Manure *for* Manure | No manure.

Page 276, paragraph 4, line 2, *insert* in connection with proper soil moisture after good soil treatment.

Page 488, line 4, *read* Two *for* the.

Page 502, Entry 37, *omit* repeated matter.

Page 502, Entry 48, *read* potatos *for* potatoes [so in original].

Cover of July number, *read* E. F. Gaines *for* E. E. Gaines.

Page 572, legend for Fig. 4, *read* old culture *for* cold culture.

Page 664, line 1, *after* barley *insert* rust.

Page 676, Table 1, in column headed "Solution," opposite last entry for April 20, *read* emulsin *for* emulsion.

Page 699, line 8, *read* 10 gm. *for* 10 m.

Page 706, Table 1, under *U. macrosperma* *delete* *P. pubescens*.

Page 708, before last paragraph *insert* Note 9.

Page 832, line 16, *read* active *for* alive.

Page 839, line 26, *read* pure *for* parts.

Cover of November number, *read* H. K. Chen *for* K. H. Chen.

Page 919, line 21, *read* 36.3 *for* 26.6.

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To

Erwin Frink Smith

scientist , linguist , poet , friend,
who for forty years has devoted
his life's service to the
broad field of pathology ,
in grateful appreciation
we the members of the
American Phytopathological Society
dedicate this testimonial.

 or leadership in early study of peach yellows,
most stimulating example of dogged work
upon a baffling problem, with prophetic
assurance that knowledge of tobacco mosaic and
aster yellows was pertinent to the solution;

For leadership in pioneer studies of
bacterial plant pathogens, with classic publications
exacting models for all who followed; again with
prophetic vision of the boundless extent of this
field;

For zealous devotion in defense of truth;

For assembled contributions to knowledge
of bacteria in relation to disease in plants;

For epochal researches on crown gall;

For sympathetic counsel to eager younger
scientists, from far and near;

For thus exemplifying the Pasteurian
characteristics: clear vision, instant action,
intuitive judgment, precise method, tireless
endeavor, sympathetic patience, self-sacrificing
devotion in service through science;

For these things we delight to honor you:

Pioneer, prophet, exemplar, dean of our
science.






No one in our day has done more to bring the two great divisions of pathology in close relation to their mutual advantage.

Your studies of plant tumors have brought you into the field of oncology in its broadest aspect. Here you take your place in national and international congresses and associations devoted to medical research and here your work is recognized as of the greatest interest and importance.

While your name is associated especially with the championship of the parasitic theory of the origin of tumors, your studies of the mechanism of tumor formation, of problems of histogenesis, of formative stimuli and inhibition of growth are scarcely of less importance.

We too on the medical side have learned to admire you as a man inspired with the highest ideals of the searcher for truth, and devoted to this search, with the heart, the methods and the loyalty of the ideal man of science.

William H. Welch

hat Robert Koch was to the early days of human and animal bacteriology, that and more have you meant to the bacteriology of plant diseases. Almost single handed you saw it through those first years of speculation and scepticism to its present broad and solid position among the sister sciences.

In your scientific work and in your influence you have made an indelible impression not alone upon plant science or upon animal science, but upon the whole field of experimental biology. And what is to me most vital and reassuring, through it all you have never for a moment lost sight of the humanities nor of the beautiful things of the mind and of the world without.

May I therefore be permitted to add the personal tribute of one who for over fifteen years has worked under the inspiration of your guiding hand.

Frederick V. Rand

At the eighteenth annual meeting of the American Phytopathological Society, held in Philadelphia, December twenty-ninth, Nineteen Hundred and Twenty-Six, in connection with the winter convocation week of the American Association for the Advancement of Science, a banquet was given in honor of Dr. Erwin F. Smith, dean of American phytopathologists. Following the dinner, addresses were made summarizing the manifold phases of the life and work of this most unusual man; and as a testimonial from the Society at large, a leather-covered brochure, hand-engrossed on parchment paper, was presented, which included a dedicatory statement, summaries of the addresses, and signatures of members of the Society and visiting pathologists. In view of the untimely death of Dr. Smith it seems fitting that on the anniversary of this meeting the Society place this testimonial on permanent record for its members.

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BIOMETRICAL STUDIES ON THE VARIATION OF PHYSIOLOGICAL FORMS OF *PUCCINIA GRAMINIS TRITICI* AND THE EFFECTS OF ECOLOGICAL FACTORS ON THE SUSCEPTIBILITY OF WHEAT VARIETIES¹

MOSES N. LEVINE²

INTRODUCTION

The question whether specialized forms of *Puccinia graminis* Pers. are plastic or constant is not only of considerable scientific interest but also of paramount economic importance. Breeding for rust resistance can be successful only in so far as the different rust forms will maintain their stability.

¹ Presented to the Faculty of the Graduate School of the University of Minnesota as a thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy, granted December 18, 1924. Cooperative investigations between the Bureau of Plant Industry of the United States Department of Agriculture and the Agricultural Experiment Station of the University of Minnesota. The uniform rust nursery experiment in this investigation was started in 1919 by the Office of Cereal Crops and Diseases in cooperation with various state agricultural experiment stations at the instance of Drs. H. B. Humphrey, C. E. Leighty, and E. C. Stakman. In 1920, the cooperation of the Dominion of Canada Department of Agriculture was secured for extending the experiment to the grain-growing region of Western Canada.

² Associate Pathologist, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. The writer takes pleasure in acknowledging his indebtedness to Dr. E. C. Stakman, Professor of Plant Pathology, University of Minnesota, and Pathologist, Bureau of Plant Industry, United States Department of Agriculture, at whose suggestion the research was undertaken, for the stimulating encouragement and helpful criticism he afforded during the progress of the investigation. Messrs. J. Allen Clark, Agronomist, and John H. Martin, formerly Associate Agronomist, Western Wheat Investigations, Office of Cereal Crops and Diseases, U. S. Department of Agriculture, the writer is indebted for having grown the seed for the uniform rust nurseries at Moccasin, Mont., and for kindly distributing it to the different cooperating stations. The writer is under obligation to Professor W. P. Fraser, formerly Pathologist in Charge of Cereal Disease Investigations in the Dominion of Canada Department of Agriculture and to the workers at the different agricultural experiment stations in the United States under whose auspices the uniform rust nurseries were maintained, for their excellent cooperation and numerous courtesies. Sincere thanks also are due Dr. J. Arthur Har-

i.e., so long as they will not "adapt" themselves easily to new varieties and will not be readily influenced by changing conditions. What rôle environmental conditions play in "predisposing" cereal crops to the attack of the stem-rust fungus is another serious question that deserves the careful consideration of agronomists as well as phytopathologists.

Extensive studies have been made by various workers on the effect of host plants and other factors on the parasitic capabilities and the morphologic structures of different forms of stem rust. [See Freeman and Johnson (11), Stakman and Piemeisel (35), Stakman and Levine (29, 30), Newton (20), and Levine (15) for literature reviews and original research on this subject.] These studies have been confined, however, almost entirely to the composite group-forms, or varieties, of *P. graminis*.³ In recent years certain knowledge has been gained concerning the intricate nature of a few of the component strains, or physiologic forms, of the *tritici*, *avenae*, and *secalis* varieties (20, 30, 13, 3, 16, 23, 32, 39, 5, 31). But as yet very little can be found in literature concerning the effects of ecological factors on the predisposition of cereal crops to the ravages of stem rust; and still less regarding the fixity and comparative morphology of the physiologic forms comprising the different varieties of *Puccinia graminis*.

The varieties of *Puccinia graminis* Pers. now known to be endemic on the North American continent are as follows:⁴

1. *P. graminis tritici* Erikss. and Henn.; some 40 physiologic forms are known (30).
2. *P. graminis secalis* Erikss. and Henn.; about a dozen separate forms are known (16).
3. *P. graminis avenae* Erikss. and Henn.; five physiologic forms have been described (32, 5).
4. *P. graminis phleipratensis* (Erikss. and Henn.) Stak. and Piem.; has not been separated into forms (35).
5. *P. graminis agrostis* Erikss.; has not yet been separated into forms (35).

Head, Department of Botany, University of Minnesota, Dr. Clyde E. Leighty, Agronomist, Office of Cereal Crops and Diseases, U. S. Department of Agriculture, and Dr. Holbrook Working, formerly Associate Professor, Agricultural Economics, University of Minnesota, for valuable suggestions pertaining to the statistical data presented in this paper. Finally, to Dr. C. R. Ball, Senior Agronomist in Charge, and to Drs. H. B. Humphrey and A. G. Johnson, Senior Pathologists, Office of Cereal Crops and Diseases, U. S. Department of Agriculture, the writer is under obligation for reading and editing this manuscript.

³ In conformity with the resolution recently adopted by the American Phytopathological Society conjointly with the American Society of Agronomy and the Mycological Section of the Botanical Society of America (4), the term "variety" is hereinafter used to designate the composite group-forms of *P. graminis*; the term "form," or "physiologic form," refers to a specialized race within a given "variety"; Arabic numerals are employed to designate the several physiologic forms within a given variety.

⁴ For list of host plants see Stakman and Piemeisel (35), Stakman and Levine (31), and Bailey (5).

6. *P. graminis poae* Erikss. and Henn.; existence of physiologic specialization has not definitely been established (31).

Historical Review

It has been commonly observed that the prevalence and severity of epidemics of stem rust depend not only on the presence of sufficient quantities of aecial or uredinial material in the spring, but also on meteorological conditions favorable for infection, such as proper temperature, abundant moisture, and sufficient sunshine. Bolley (7) states that dew and cool temperature favor rust infection. In Carleton's opinion, the prevalence of the disease is dependent largely on the humidity of the atmosphere (9). According to Butler and Hayman (8), "moisture as indicated by cloud and, to a lesser extent, an excess of water in the soil induce rusting of the crop." Sorauer (25) states that sharp differences between clear cold nights and hot days with abundant dew are especially favorable for rust infection. Freeman and Johnson (11) thought that the "unusually low temperature in 1904 was a very important factor, if not the determining factor, for the rust epidemic of that year."

Stakman and Levine (29) found that the optimum atmospheric temperature for the development of stem rust on wheat in the greenhouse ranges between 66.5° and 70.0° F. [about 19° to 21° C.]. They also found that a sufficiency of moisture and plentiful light are indispensable for the best growth of the rust. A detailed greenhouse study with two physiologic forms of *P. graminis tritici* led Peltier (23) to conclude that the optimum temperature for the initial infection of, and subsequent development on, susceptible varieties "in the seedling, stooling, and jointing stages was between 20° and 25° C. [68°-77° F.]. No infection occurred at temperatures of 10° C. [50° F.] and below, while only a few plants of some differential hosts were infected at 15° C. [59° F.] and 30° C. [86° F.]. With plants at the heading stage a lower optimum temperature for infection occurred in that no rust developed at 30° C. [86° F.], while it did at 10° C. [50° F.]." Tehon and Young (37) are of the opinion that a mean temperature of 71.5° F. may be considered as indicating the probable optimum for stem-rust infection under field conditions in Illinois. They also hold that there is an apparent correlation between periods of precipitation and the occurrence of infections. Beauverie (6) ascribes the absence of stem rust from the wheat varieties grown at Limagne and Clermont, France, in 1921, to the fact that there was no rain there until harvest time. In 1922 the rainfall at these localities was frequent and often abundant, and *Puccinia graminis* "became more and more prevalent as the season advanced." These observations led Beauverie to the conclusion that "*P. graminis* is essentially the rust of wet seasons."

Marchal (17) states that, besides weather conditions, the following factors favor the appearance of rust, *viz.*, soil moisture, stiff soil, shade, late sowing, and misuse of fertilizers. Vavilov (38) reported that neither early nor late sowing, nor the application of nitrogenous fertilizers to the soil exerted any influence on the infection capabilities of *Puccinia triticina* Erikss. Resistant varieties remained resistant under these conditions, and the reaction of susceptible varieties likewise was unaltered. Stakman and Aamodt (27), on the basis of their observations and experiments covering a period of eight years, concluded that, "while the direct effect of fertilizers on the development of stem rust seems to be slight, there is sometimes a profound indirect effect. The stem-rust attack sometimes is more severe on plants grown on soil heavily fertilized with nitrogenous manures." This, they believe, is due to the increased density of stand and delayed maturity, which make conditions for infection more favorable on account of close proximity of the plants on one hand, and the lengthening of the period during which the plants could become infected on the other. However, they found that the inherited tendency to resistance or susceptibility in a variety could not be changed fundamentally by the application of fertilizers.

Hursh (14) showed that the mycelium of *P. graminis* within the stem of the host is limited almost entirely to the chlorenchymatous tissue; and, as the only important chlorenchymatous tissue of the stem is the collenchyma, the rust mycelium can grow only in this tissue. Furthermore, he found that excessive fertilization with nitrogen has a tendency to decrease the amount of sclerenchyma in proportion to the amount of collenchyma, and, for this reason, plants heavily fertilized with nitrogen may be more severely injured by rust than those which have not been so fertilized. Weiss (40) states that "the addition of NaCl or NaH_2PO_4 to the basic three-salt nutrient solution did not affect the susceptibility of wheat to leaf rust or stem rust. The addition of NaNO_3 resulted in somewhat readier infection in each case but did not predispose to greater injury. KCl retarded infection in proportion to the diminution of growth of the host, particularly when used in excess. CaCl_2 and MgCl_2 appeared to induce a state in which the host was less readily susceptible to infection. CaCl_2 also resulted in a reduction of water requirement, about 10 per cent for tops and 40 per cent for grain." Stakman, Parker, and Piemeisel (34) observed seasonal fluctuations in the severity of infection of stem rust, but their experimental results and field observations indicate that rust resistance is a rather constant genetic character and is not controlled primarily by seasonal conditions, soil type, geographical location, or other cultural conditions, but the resistance of grain varieties may and does vary in different regions because of the presence of different physiologic forms of the rust pathogene.

In addition, then, to the presence of sufficient inoculum and the prevalence of favorable environmental conditions for infection, even on susceptible sorts, the presence of particular physiologic forms of *P. graminis tritici* is necessary for the development of epidemics on certain varieties of wheat. Stakman, Levine, and Leach (33), after having discovered the first dozen forms of *P. graminis tritici*, came to the conclusion that "the fact that the same variety of wheat may be immune in one locality and susceptible in another is clearly explained. Formerly recourse was taken to the theory that environmental conditions changed the physiologic processes and materials of wheat varieties so fundamentally that the resistance of the plants broke down. The real explanation of this phenomenon, however, is the fact that there are many biologic forms of the rust fungus." Hayes and Stakman (12), therefore, hold that further studies are necessary to determine the number and prevalence of the physiologic forms of *P. graminis tritici* which should be cultured and used experimentally in definite attempts to produce wheat varieties resistant to all forms occurring in the wheat-growing regions. Consequently a knowledge of the constancy in the parasitic behavior of these forms is obviously one of the outstanding desiderata connected with such studies.

Ever since Eriksson (10) first discovered the existence of the group-forms, or varieties, of *P. graminis* there has been a good deal of speculation as to the degree of fixity of these forms. The literature dealing with this subject has been rather fully reviewed by Stakman, Piemeisel, and Levine (36). It shall, therefore, suffice to summarize here briefly the experimental results of the latter investigators. Successive transfers of *P. graminis tritici*, *P. graminis avenae*, *P. graminis phleipratensis*, and *P. graminis agrostis* to resistant hosts did not increase their virulence. There was no indication of their gradual adaptation to resistant or semi-congenial hosts, although plus and minus fluctuations occurred. The pathogenicity of the forms did not seem to be changed either easily or permanently by host influence. No one so-called "bridging host," nor any combination of such hosts, enabled any of these rust varieties to attack naturally immune hosts nor to infect more readily highly resistant plants.

Although neither host plants nor cultural conditions seem to affect the genetic nature of the different rust varieties, Stakman and Levine (29) observed that resistant varieties and adverse environment invariably tend to decrease the size of the uredinia and urediniospores of the stem-rust fungus. However, as soon as the unfavorable condition of host and environment are removed, the spores regain their normal size in a single generation. Levine (15) later found that the aeciospores and teliospores of the different rust varieties responded to unfavorable conditions in much the same manner as the urediniospores.

These variations within a given variety of *P. graminis* are not to be confused with the inherent morphologic differences between the various group-forms *per se*. Stakman and Levine (29, 31) found a consistent difference in the color, shape, and size of the urediniospores of all the varieties of *P. graminis*, when produced under uniform conditions. Levine's (15) subsequent statistical studies show that these varieties differ distinctly and significantly in the size of aeciospores and teliospores as well as urediniospores. In general, *P. graminis tritici* has the largest spores; *P. graminis avenae* occupies the second place; *P. graminis secalis*, third; *P. graminis phleipratensis*, fourth; *P. graminis agrostis*, fifth; and *P. graminis poae* has the smallest spores of all.

Stakman and Levine (29) found that the group of physiologic forms to which the hard red spring wheat varieties as a class are highly resistant possessed urediniospores which were consistently, though not very greatly, smaller than the urediniospores of the group of forms which parasitizes the hard red spring wheats very readily. Waterhouse (39) detected morphological differences in the two physiologic forms of the wheat stem-rust fungus which he discovered in Australia. He found that his form 1 has a mean length of more than one micron less than form 2, but the mean width of the first form is nearly four microns greater than that of the second form. He also states that "a casual examination of the uredospores gives the impression that the average spore of form 2 is more slender than that of form 1." In addition to differences in parasitism, Bailey (5) observed significant differences in the size of the urediniospores of the several physiologic forms of *P. graminis avenae* when all were grown under identical conditions.

Hursh (13) demonstrated distinct differences between *P. graminis tritici* 11 Stak. and Lyne. and *P. graminis tritici* 27 Stak. and Lyne. with respect to their germination response to extremes of hydrogen-ion concentration and temperature. Allen (3) found but little difference between forms 3 and 19 Stak. and Lyne. in their effect upon the stomata of wheat varieties. On the whole, form 3 affected Baart wheat more strongly, but the difference in the action of the two forms on the stomata of Kanred and Mindum was hardly perceptible, although the two rusts differ greatly in their ability to parasitize these varieties.

In view of all these facts it seemed desirable to make more systematic and extensive observations in the field concerning the effects of ecological factors on the severity of stem-rust epidemics, and to conduct more intensive studies in the greenhouse and laboratory to obtain further evidence relative to the idiosyncrasies and the stability of the physiologic forms of *P. graminis tritici*.

Purposes of Investigation

The purposes of the present investigation were:

1. To determine the degree of resistance to stem rust of varieties of spring wheat and emmer in the wheat-growing regions of the United States and Canada, under natural climatic and edaphic conditions.
2. To study the rust reaction of these varieties in the field in the presence of physiologic forms of *Puccinia graminis tritici*, capable of attacking them readily in the greenhouse, and *vice versa*.
3. To ascertain the number and distribution of physiologic forms of *P. graminis tritici* in different parts of the spring wheat region.
4. To determine the identity and nature of these forms by means of artificial inoculations on differential varieties of *Triticum spp.*
5. To determine by means of statistical studies the comparative morphology of some of these forms.
6. To determine the effect of prevailing weather conditions, condition of host plant, amount of inoculum, diverse cultural practices, etc., on the severity of infection of stem rust on wheat in the field.
7. To study the constancy of these forms under varying cultural conditions, and the effect of geographical location and host plant on their pathogenicity and stability.

EXPERIMENTAL MATERIALS AND METHODS

The nature of the present investigation was twofold: first, observational; and second, experimental. The observations were made over a period of five years on a series of uniform rust nurseries grown in different localities. The experimental phase consisted of greenhouse and laboratory studies, some of the experiments having been conducted for as long as six and nine years.

Uniform Rust Nurseries, Names of Cooperators, Varieties Tested

A series of nurseries, each consisting of a uniform set of wheat varieties, was established at various points in the United States and Canada (See figure 1 and table 1). These nurseries are hereinafter designated as uniform rust nurseries. The first year the uniform rust nurseries were maintained at 13 different experiment stations in the United States (Table 3). At some of the stations the nurseries were abolished the following year; however, several new stations were added in the United States and a similar uniform nursery experiment was started at eight stations in Canada (Table 4). In 1921 the uniform rust nurseries were in operation at 14 experiment stations in the United States and at 10 in Canada (Table 5). In 1922 and 1923 uniform rust nurseries were operated at 19 experiment stations in the United States, the cooperative experiment in Canada having been discontinued after 1921 (See tables 6 and 7). At St. Paul, Minn., there were two uniform rust nurseries during the last three years of the experiment (1921-1923). In one, the rust epidemic was induced arti-

ficially using several physiologic forms as inoculating material, in the other the infection was permitted to come about naturally. In 1923 there were two nurseries also at Coon Creek, Minn. one on a sand hill and one in a peat bog.

Seed for the uniform nurseries was grown under rust free conditions at Moccasin, Montana, and was furnished the cooperators at the various experiment stations by the western wheat investigations project of the Office of Cereal Crops and Diseases, U. S. Department of Agriculture, each year, except 1920. In that year the cooperators used the seed they obtained from the 1919 harvest of their respective nurseries.

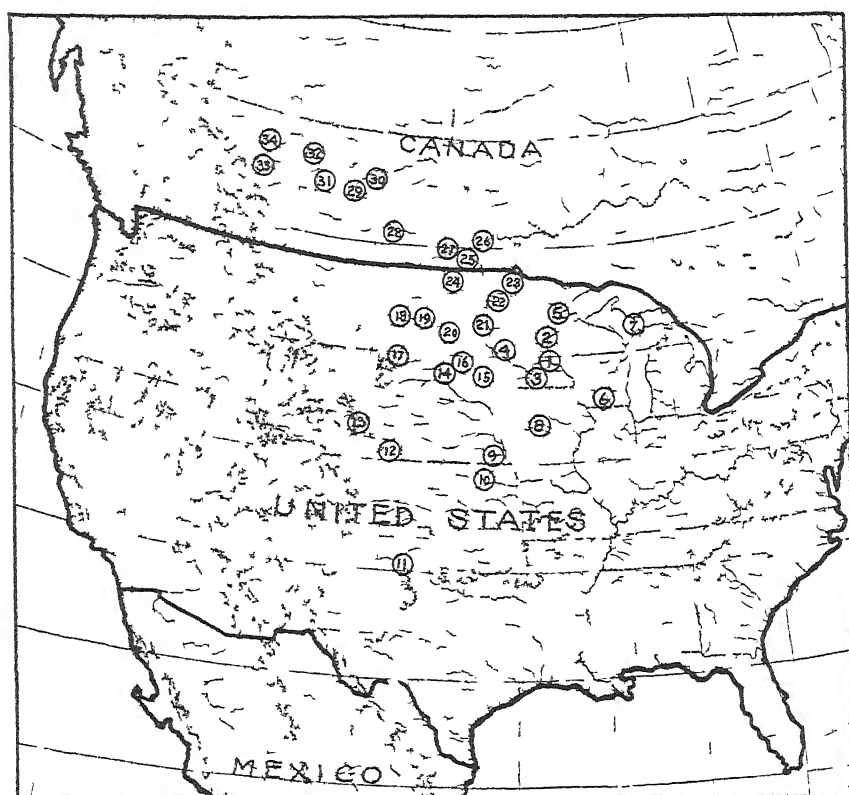


FIG. 1. Location of uniform rust nurseries at 34 experiment stations in the United States and Canada during one or more years of the five year period, 1919-1923, inclusive.

The maximum number of varieties grown during any one season in the course of the present experiment was 24. Some were known to be generally resistant to stem rust under field conditions *e.g.*, Acme, Monad, Pentad, and

TABLE 1.—Location of uniform rust nurseries in the United States and Canada, and names of cooperators at each station

Map no.	Experiment station	Cooperators
1	St. Paul, Minn.	H. K. Hayes, O. S. Aamodt
2	Coon Creek, Minn.	G. H. Nesom
3	Waseca, Minn.	R. E. Hodgson
4	Morris, Minn.	R. O. Bridgford
5	Duluth, Minn.	M. J. Thompson, H. C. Gilbert
6	Madison, Wis.	J. G. Dickson
7	Chatham, Mich.	G. H. Coons, J. E. Kotila
8	Ames, Iowa	S. M. Dietz
9	Lincoln, Nebr.	T. A. Kisselbach
10	Manhattan, Kans.	L. E. Melchers, J. H. Parker
11	Amarillo, Tex.	J. F. Ross
12	Akron, Colo.	F. A. Coffman
13	Archer, Wyo.	A. L. Nelson
14	Highmore, S. Dak.	E. S. McFadden
15	Brookings, S. Dak.	A. T. Evans, Matthew Fowlds
16	Redfield, S. Dak.	Samuel Garver
17	Newell, S. Dak.	A. D. Ellison
18	Dickinson, N. Dak.	R. W. Smith
19	Mandan, N. Dak.	J. C. Brinsmade, Jr.
20	Edgeley, N. Dak.	W. E. Brentzel, O. A. Thompson
21	Fargo, N. Dak.	W. E. Brentzel
22	Crookston, Minn.	R. S. Dunham, E. R. Clark
23	Golden Valley, Minn.	G. H. Nesom
24	Langdon, N. Dak.	W. E. Brentzel, Louis Jorgenson
25	Morden, Manitoba	W. P. Fraser
26	Winnipeg, Manitoba	
27	Brandon, Manitoba	
28	Indian Head, Saskatchewan	
29	Saskatoon, Saskatchewan	
30	Rosthern, Saskatchewan	
31	Scott, Saskatchewan	
32	Vermillion, Alberta	
33	Lacombe, Alberta	
34	Edmonton, Alberta	

Kota; others were included because of their early maturity, *e.g.*, Prelude and Ruby; and still others were important commercial varieties in the United States and Canada, as, for example, Marquis, Power, Kubanka, Arnautka, and Mindum. Varieties which are essential in the determination of the physiologic forms of *P. graminis tritici*, such as Kubanka durum (C. I. 2094), Arnautka ("Speltz Marz") durum (C. I. 6236), and Vernal (White Spring) emmer (C. I. 3686) also were included. Little Club, which is completely susceptible, and Khapli, which is extremely resistant to all

known physiologic forms in North America, were added because of their contrasting differences. The varieties used in one year or another are listed in table 2.

A physiologic form of *P. graminis tritici* has been found recently to which the behavior of Little Club and Khapli emmer has been reversed. This form was isolated from a rusted specimen of Federation wheat grown at Giza, Egypt. It attacks Khapli rather severely in both the seedling and post-heading stage; Little Club shows considerable resistance in all stages of development, especially under deficient light conditions.

TABLE 2.—*Varieties of wheat and emmer grown in uniform rust nurseries to determine their reaction to stem rust under field conditions*

Class and variety	Cereal investigations accession number
Durum (<i>Triticum durum</i> Desf.):	
Kubanka	1440
Do	2094
Do	4063
Arnautka	1493
Do	4064
Do "Speltz Marz"	6236
Mindum	5296
Acme	5284
Monad	3320
Pentad	3322
Kahla	5529
Peliss	1584
Hard Red Spring (<i>Triticum vulgare</i> Vill.):	
Haynes Bluestem	2874
Marquis	3641
Power Fife	3697
Ruby	6047
Kitchener	4800
Red Bobs	6255
Preston	3081
Kota	5878
Prelude	4323
White Club (<i>Triticum compactum</i> Host.):	
Little Club	4066
Emmer (<i>Triticum dicoccum</i> Schrk.):	
Vernal (White Spring)	3686
Khapli	4013

In 1919 and 1920 only 18 of the 24 varieties enumerated in table 2 were grown. These included the three strains of Kubanka, two strains of Arnautka (C. I. 4064 and C. I. 6236) and Mindum, Acme, Monad, and

Pentad of the durum class; all of the hard red spring varieties listed, except Kitchener and Red Bobs; and both of the emmer varieties. In addition to these, two hybrids were included, namely, Kubanka \times Haynes (C. I. 4788) and Kubanka \times Preston (C. I. 4789). In 1921 and 1923 the complete set as given in table 2 was used. In 1922 Kubanka (C. I. 2094) and Arnautka (C. I. 6236) were omitted on account of lack of seed.

In 1919 and 1920 each variety was grown in three adjacent rod rows. The rate of seeding was 16 grams per row for wheat and 20 grams for emmer. Duplicate sowings were made at each station at an interval of about two weeks. Beginning with 1921 the sowing of each variety was reduced to two rod rows instead of three and to a single seeding instead of two.

Inoculation Technique and Cultural Methods in the Greenhouse

Specimens of fresh uredinial material were collected at the various uniform rust nurseries at the time the rust estimates were made. The material was sent to University Farm, St. Paul, where it was used promptly to inoculate as many of the differential varieties as possible. The inoculation and incubation methods followed were essentially the same as those described by Stakman and Piemeisel (35). Proper precautions were taken to reduce the amount of accidental infection to a minimum. The seedlings of the differential hosts were grown in a secluded section of the greenhouse. Immediately after inoculation the seedling plants were placed in galvanized iron incubation chambers, containing water to the depth of approximately one inch, and covered with glass frames. After 48 hours the inoculated plants were removed from the incubation chambers and placed in booths on the greenhouse benches. Partitions consisting of two layers of thick muslin separated the booths. Rust notes were taken 12 to 18 days later, depending on the season of the year and prevailing weather conditions. When all the differential varieties were tested and their reactions recorded, the identity of the physiologic forms in question was determined by means of the dichotomous key of Stakman and Levine (30).

Single-spore Isolations and Spore Measurements

A number of physiologic forms employed in this study were purified by means of single spore isolations. Isolating single urediniospores is tedious and painstaking, and it was found desirable to have two men perform the task, one doing the isolating and the other the inoculating.⁵ This also afforded additional insurance of the accuracy of the procedure, for each

⁵ The writer greatly appreciates the careful and efficient assistance in the monosporous culture work of Mr. Alfred E. Eagle, greenhouse and field foreman, Division of Plant Pathology and Botany, University of Minnesota.

spore picked up was checked by the assistant. To eliminate the possibility of chance contamination, the work was done where there were no rust cultures and behind a curtain designed to arrest any wind-borne spores which might be blown in from outside. Hands were thoroughly cleansed with soap and water, and incubation chambers (galvanized iron tanks and glass covers) were first steam sterilized for twenty minutes and then allowed to cool gradually. When cool, water to a depth of one inch was put in the tank and the incubation chamber was placed in spore-proof glass compartments, in which the inoculated plants were to remain thenceforth.

Two microscopes with low power objectives were used, one by the isolator and one by the inoculator. The first microscope was provided with a mechanical stage. A special metal contrivance (hereafter to be designated as "adjuster") was affixed to the stage of the second microscope.⁶ Urediniospores of the physiologic form to be purified were scattered over a dry microscope slide by gentle tapping an infected blade of wheat. The spores were further separated with the aid of a dissecting needle prior to placing the slide in the mechanical stage. Single spores were picked up by the isolator by means of a finely pointed clean needle (Sharps 8 sewing needle) covered with a very thin film of vaseline. Upon examining the tip of the needle under the microscope and being satisfied that it bore a solitary urediniospore, it was handed over to the inoculator. The latter, without jarring it, placed the needle in the metal adjuster which was so arranged that the point of the needle was always maintained in the field of vision. While the needle was turned completely around, examination was made under the low power of the microscope. If more than one spore was detected, or if no spores could be noticed, another attempt was made to pick up a single spore.

When one spore only was adhering to the needle, the latter was removed from the adjuster by the right hand. Its point, containing the single spore, was then dipped in the tiny drop of water previously placed by means of a wooden toothpick within one-half inch from the tip of the leaf to be inoculated. The needle was then gently wiped on the leaf surface, care being taken not to injure the tissue. The leaf was kept in position by a toothpick held in the left hand, which was subsequently placed near the plant as a sign that it had been inoculated. When this was accomplished, the needle was again placed in the adjuster and examined to ascertain whether the spore had been removed. When no spore could be seen, it was assumed that the spore had been deposited on the plant. The needle

⁶ The writer wishes to express his gratitude to Mr. Gilbert D. George, of the Division of Publications of the University of Minnesota, and the United States Department of Agriculture, for designing and manufacturing this device.

was then handed back to the isolator, who in the meantime had another needle charged with a single spore ready for the inoculator. Thus both workers were kept steadily busy, without having to adjust their eyes constantly to different objects, which would necessarily have been the case had one person done both the isolating and inoculating.

After all the plants in a pot had been inoculated and marked, the pot was immediately placed in the incubation chamber and the plants sprayed from above with an atomizer containing clean tap water. The temperature in the glass cage was kept as near 70° F. as possible during the incubation period of 48 hours. At first the percentage of successful infection from the monosporous isolations was very low, 0.18 per cent. It took 547 isolations to secure the first successful infection, but when conditions were just right, three infections were once obtained from only 57 isolations. On this occasion there was as high as 5.3 per cent of successful infection, the average being about 1 per cent.

Eight physiologic forms of *P. graminis tritici*, four of monosporous origin and four stock cultures, were subjected to a statistical morphologic study. Both diameters of each urediniospore under observation were measured and recorded. All of the spore measurements were made with a Zeiss screw micrometer attached to a calibrated Spencer microscope. Unconscious selection of spores for measurement was avoided by measuring all the spores which were encountered in passing from one end of the mount to the other. It was found that in virtually every case one hundred urediniospores were amply representative of the populations studied. The statistical constants were calculated by means of the accepted formulae. The odds against the chance occurrence of the differences obtained were ascertained according to the computations of Pearl and Minor (21). The shape of the urediniospores was determined by the method suggested by Rosenbaum (24). The mean length-to-width ratios of a number of physiologic forms determined by this process were compared with the ratios resulting from dividing the mean lengths of the respective forms by their mean widths, a method employed by some workers. The probable error of the quotient of the two quantities last named was derived by the formula given by Mellor (19, p. 529), which is as follows:

$$E = \pm \frac{\sqrt{\left(\frac{Ba}{A}\right)^2 + b^2}}{A}$$

where E stands for the probable error of the quotient (B : A), respectively affected by the probable error $\pm b$ and $\pm a$.

As it was previously found (29) that the superficial layer of each uredinium contains larger spores, the urediniospores to be measured were

dusted into a drop of water on the microscope slide. In all other respects the method was the same as that in the previous experiments (29, 15).

RESULTS OF INVESTIGATION

The results of the observations on the reaction of the wheat varieties in the uniform rust nursery experiment are summarized in tables 3 to 8, inclusive. The physiologic forms isolated each year from the various nurseries are listed in table 9. Tables 10 and 11 show the behavior and distribution of these forms. The data on the morphology of certain important forms are presented in tables 12 to 40 inclusive. A detailed analysis of these results appears under the captions: "Field Observations on Severity of Rust Epidemics" and "Greenhouse and Laboratory Studies of Physiologic Forms."

Field Observations on Severity of Rust Epidemics

Previous to 1922 the rust estimates at some of the uniform nurseries were made by others than the writer. Thereafter, to obviate errors in the rust estimates, incident to differences of a personal equation, the writer whenever possible inspected all the nurseries himself, and wherever feasible took the rust readings together with the person in charge of the experiment. The scale (Fig. 2) used in the stem rust investigations made by the Office

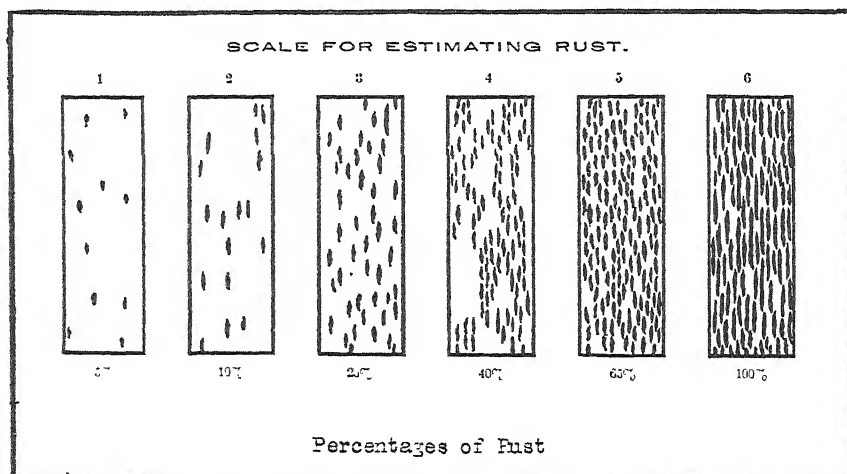


FIG. 2. Scale for estimating percentage of stem rust. The shaded spots represent rust, and the figures represent approximately the rust percentages computed on the basis of the maximum amount of surface covered by rust as shown in No. 6, which represents 37 per cent of surface covered with rust pustules and is arbitrarily selected as 100 per cent. Other percentages are based on No. 6. (From Office of Cereal Crops Diseases, United States Department of Agriculture).

of Cereal Crops and Diseases, United States Department of Agriculture, was used in estimating the percentage of rust on the varieties in the uniform nurseries. The rust estimates, as a rule, were made just before the plants ripened. However, the rust notes sometimes were taken while some of the varieties were still quite green, whereas in other cases a number of the varieties were dead ripe at the time the rust estimates were made. For obvious reasons this could not always be remedied. Whenever a variety was not grown at all of the stations, or the yearly average was not comparable with the other varieties, the fact is noted in footnotes to the tables. The percentage of stem rust infection of each variety of wheat and emmer at each uniform nursery, and the average infection of each variety for each year, are shown in tables 3 to 7 inclusive. Following the presentation of the data by years, weighted average infections for all varieties, during the entire five-year period, are given in table 8; these are then compared with the standard variety (Marquis, C. I. 3641) for the identical period of nursery years.

Rust Situation in 1919.

A heavy stem rust epidemic developed in the spring wheat region in 1919, but at St. Paul, Minn., only a trace of rust developed on all the durum varieties except Arnautka (C. I. 4064). Of the common varieties, Kota only was entirely rust free, whereas Prelude matured very early and obviously escaped the rust attack (see table 3). The identification of the physiologic forms which were responsible for the St. Paul epidemic might explain the seemingly peculiar behavior of the durums there. As will be seen from table 9, forms 3 and 12 were isolated from the St. Paul nursery in 1919. Stakman and Levine (30) have shown that Kubanka (C. I. 2094), Arnautka (C. I. 4072 and C. I. 6236), and Mindum (C. I. 5296) are all extremely resistant to form 3. The action of form 12 on the differential varieties of *Triticum* spp. is identical to that of form 3 in every respect except one—form 12 attacks Arnautka (C. I. 4072) very heavily. But Arnautka (C. I. 4064) has been found to react to the physiologic forms of *P. graminis tritici* in exactly the same manner as does Arnautka (C. I. 4072). This evidently accounts for the 65 per cent infection on Arnautka (C. I. 4064) and for the absence of infection on Arnautka (C. I. 6236), Mindum (C. I. 5296), and the three Kubanka strains. The few rusted individuals among the last named varieties evidently were "rogues."

Owing to severe drought and excessive heat at Dickinson, N. Dak., only a trace of stem rust could be found in the uniform nursery there. This was confined to three varieties: Arnautka (C. I. 6236), Mindum (C. I. 5296), and the Kubanka \times Haynes hybrid (C. I. 4788).

TABLE 2. — Percentage of stem rust on 20 varieties of spring wheat and amount on uniform nurseries at 13 experiment stations in the United States in 1919
[T = Trace]

Experiment stations	Durum wheat										Hard red spring wheat										Runner	
	Kubanka 1440	Kubanka 2094	Kubanka 4063	Armatuka 4004	Armatuka 6286	Milium 5296	Aeue 5284	Monad 5320	Pentad 5322	Hayes 5374	Margos 5041	Dover 5097	Iday 6047	Preston 5051	Wol 5575	Prelude 1823	Kubanka 7 Hayes 4788	Kubanka 7 Preston 4789	Vernal 3686	Knap 4043	T ^a	L ^b
Prarie (subhumid):	L ^a	L	L ^a	59	L ^a	L ^a	L	L	0	50	100	60	52	100	0	L	30	100	0	L		
St. Paul, Minn.	15	15	40	60	40	L ^a	10	10	0	100	100	100	..	100	0	..	100	100	0	0	0	0
Fargo, N. Dak.	10	25	65	65	65	59	0	0	0	0	55	100	..	95	5	5	100	100	0	0	0	0
Brookings, S. Dak. ..	15	5	65	50	25	5	5	5	L	100	85	90	65	90	95	59	100	100	0	0	0	0
Lincoln, Nebr.	15	5	65	50	25	5	5	5	L	100	85	90	65	90	95	59	100	100	0	0	0	0
Ames, Iowa	25	15	d.	30	10	5	L	5	5	40	75	85	..	65	L	..	90	100	0	0	0	0
Manhattan, Kans. ...	65	15	d.	30	10	5	L	5	5	40	75	85	..	65	L	..	90	100	0	0	0	0
Great Plains (semi arid):	7	25	40	15	25	15	7	5	L	10	55	37	15	13	0	10	20	15	0	0	0	0
Mandan, N. Dak.	25	25	65	65	80	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dickinson, N. Dak. ..	25	65	65	65	80	5	5	5	0	100	70	95	20	90	L	15	40	25	1	1	1	1
Illigmore, S. Dak. ...	5	5	5	5	25	5	20	5	5	10	50	40	65	10	5	25	50	65	5	5	5	5
Nowell, S. Dak.	15	25	35	10	50	65	10	10	5	65	65	65	10	65	L	15	35	50	5	5	5	5
Archer, Wyo.	10	8	10	8	8	12	5	1	0	35	15	15	20	25	L	15	10	10	0	0	0	0
Akron, Colo.	10	10	10	15	10	12	L	L	0	65	65	55	70	70	1	60	40	25	0	0	0	0
Amarillo, Texas	14	13	26	34	50	18	5	4	1	66	57	62	59	66	3	27	41	42	24	1	1	1
Average	14	13	26	34	50	18	5	4	1	66	57	62	59	66	3	27	41	42	24	1	1	1

^a A few but normal pustules.

^b Mostly leaf-rust infection.

^c Glumes and awns rusted.

^d Harvested before rust notes were taken.

^e Average of only 12 nurseries.

^f Average of only 11 nurseries.

TABLE 4.—Percentage of stem rust on 20 varieties of spring wheat and durum grown in uniform nurseries at 16 experiment stations in the United States and Canada in 1920
[T = Trace]

Experiment stations	Estimated percentage of infection on													
	Durum wheat							Hard red spring wheat						
	Kubanka 1440	Kubanka 2094	Kubanka 4063	Arnavka 4064	Arnavka 6236	Mindum 5206	Ame 5284	Monad 3220	Pentad 3222	Haynes 2874	Marguis 3041	Power 3697	Ruby 6047	Preston 3081
UNITED STATES														
Prairie (subhumid):														
St. Paul, Minn. ^a	95	100	56	95	95	95	25	55	0	100	63	100	40	100
Fargo, N. Dak.	15	15	15	40	40	40	10	55	4	100	59	52	40	100
Lincoln, Nebr.	10	10	15	40	40	40	10	55	0	100	59	52	40	100
Great Plains (semiarid):														
Mandan, N. Dak.	15	15	20	25	20	15	1	1	1	80	65	65	40	40
Dickinson, N. Dak.	10	6	7	5	5	5	7	5	4	40	40	40	40	40
Highmore, S. Dak.	5	5	5	5	5	5	5	5	5	40	40	40	40	40
Archer, Wyo.	25	25	25	50	50	50	10	15	1	50	50	50	50	50
Akron, Colo.	45	35	45	40	35	35	10	15	1	50	50	50	50	50
CANADA														
Morden, Manitoba	25	25	30	25	25	25	7	7	1	50	50	50	50	50
Winnipeg, Manitoba	40	40	40	50	50	50	25	25	2	65	50	50	50	50
Brandon, Manitoba	40	50	40	50	50	50	25	25	2	65	50	50	50	50
Indian Head, Saskatchewan	30	35	40	35	40	35	25	25	2	65	50	50	50	50
Saskatoon, Saskatchewan	30	40	30	30	30	30	10	7	1	40	40	40	40	40
Rosthern, Saskatchewan	35	40	45	35	35	35	10	7	1	40	40	40	40	40
Scott, Saskatchewan	8	2	2	1	1	0	0	1	0	35	25	25	20	25
Edmonton, Alberta	2	2	3	1	1	0	0	1	0	35	25	25	20	25
Average	27	32	37	33	33	25	10	10	3	53	41	47	41	54

^a Nursery inoculated with spores of several physiologic forms found in Minnesota.^b Not grown; percentage of rust estimated from Poyer (C. I. No. 3697).^c Not grown; percentage of rust estimated from Arnavka (C. I. 4064).^d Not grown; percentage of rust estimated from Kubanka (C. I. 1440).

The agreement between the reaction of the varieties in the field and the greenhouse usually was fairly close. However, Acme, Monad, Pentad, and Kota constituted an exception to this rule. The resistance of these varieties in the field was striking, although the rust forms isolated from the nurseries readily attacked these varieties in the greenhouse. Otherwise, all varieties reacted the same in the field as they did in the greenhouse to cultures of the physiologic forms isolated from the plots in which they were grown.

It will be observed from tables 9 and 11 that seven different physiologic forms were isolated from eight of the 13 nurseries grown in 1919. At least two physiologic forms were responsible that year for the epidemics at Brookings, S. Dak. (forms 3 and 5); Fargo, N. Dak. (forms 3 and 22); and St. Paul, Minn. (forms 3 and 12). It will be noted further that form 3 appeared at all of these stations. This form also was found at Ames, Iowa, and thus was the most common physiologic form of *P. graminis tritici* in the spring-wheat region in 1919. Form 17 was found at but two stations: Akron, Colo., and Mandan, N. Dak. The other five forms were obtained from a single station each.

Rust Situation in 1920.

In 1920 there was another epidemic of stem rust. Acme, Monad, Pentad, and Kota rusted much more severely than in the previous year. It will be seen from table 4 that the highest percentage of infection on Pentad was found at St. Paul, namely, 30 per cent. At Brandon, Manitoba, 50 per cent infection was recorded on Kota, 30 per cent on Acme, and 25 per cent on Monad. At St. Paul, the rust infection on Kota was 40 per cent, whereas in the previous year Kota was totally free from rust. A difference in the weather conditions at the critical period apparently was responsible for the difference in the reaction of this variety to the rust attack. Physiologic forms virulent on Kota in the greenhouse were used in creating the rust epidemics at St. Paul in both years. The forms isolated in both years infected Kota heavily in the greenhouse.

Vernal was practically free from rust in 1920 at all stations except at St. Paul, where a virulent form was used for producing the epidemic. Professor Fraser reported that Vernal rusted heavily at Saskatoon, Sask., in 1919. But form 9, which attacks Vernal normally, was prevalent there that year, whereas in 1920 this form was late in appearing.

Form 17 led the frequency list in 1920, having appeared at five experiment stations. Form 3 followed as a close second with an invasion of four localities. In general, the 1920 epidemic in the uniform rust nurseries seems to have been caused by practically the same physiologic forms as in

1919 (table 9). Eight forms were isolated from 10 of the 16 stations in the United States and Canada (table 11).

Rust Situation in 1921.

In general, the stem rust epidemic in the uniform nurseries was somewhat milder in 1921 than in the two previous years. Table 5 shows that in the agronomy nursery at St. Paul only a trace of rust developed on a few of the durum varieties and only moderate infection on most of the hard red spring wheats. The plants matured early and evidently escaped the rust attack. A similar situation prevailed at Morris, Minn. At Golden Valley, Minn., and at Mandan and Dickinson, N. Dak., there was a very weak rust infection even on the most susceptible varieties. Only a trace of rust was present at Akron, Colo., where the weather conditions during the growing season were particularly unfavorable for the development of stem rust. However, in Manitoba and in most of Saskatchewan stem rust was quite prevalent; but at Scott, Sask., and in the nurseries in Alberta there was almost none.

Collections of rust from 16 of the 24 nurseries in the United States and Canada were cultured and identified in the greenhouses at University Farm, St. Paul. From the pathology nursery at St. Paul, where several physiologic forms were used to induce an artificial epidemic and where seven collections were made from different varieties and crosses of wheat, six forms were isolated. These were as follows: *P. graminis tritici* forms 1, 17, 18, 29, 30, and 36, all of which, except 36, had been used for the artificial inoculation. Two physiologic forms were found at each of the following nurseries: Fargo, N. Dak. (forms 21 and 29), Edgeley, N. Dak. (forms 9 and 37), Brandon, Man. (forms 9 and 29), Edmonton, Alta. (forms 9 and 17), Rosthern, Sask. (forms 29 and 32), and Winnipeg, Man. (forms 17 and 32). Only one form was isolated from each of the following places: Crookston, Minn.; Duluth, Minn.; Brookings, S. Dak.; Madison, Wis.; Ames, Iowa; Langdon, N. Dak.; Morden, Man.; and Saskatoon, Sask.; namely, 9, 21, 17, 37, 30, 21, 29, and 24, respectively.

It is possible that more forms than those isolated were actually responsible for the rust infection at the various stations in 1921. This probably accounts for some of the discrepancies in the behavior of the varieties in certain nurseries and the reaction of the differential hosts in the greenhouse toward the physiologic forms obtained from them. On the whole, however, there was a general agreement between the field observations and the results of inoculations in the greenhouse.

Rust Situation in 1922.

The infection in the nurseries was heavier in 1922 than in any of the other years during the course of the investigation (See table 6).

TABLE 5.- *Percentage of stem rot on 24 varieties of spring wheat and summer grown in uniform nurseries at 24 experiment stations in the United States and Canada in 1921*
[P. Trace]

Experiment Stations	Durum wheat												Hard red spring wheat										Club wheat	Summer
	Kubanka 1440	Kubanka 2094	Kubanka 4063	Arbutka 1493	Arbutka 4064	Arbutka 6536	Madam 3296	Avena 2984	Monard 3320	Pentard 3322	Kahla 3320	Pelika 1354	Haynes 2874	Marquis 3041	Power 3007	Indy 4047	Kitchener 4800	Red Robs 6233	Pre-Don 3081	Kota 3578	Pringle 4323	Little Club 4076		
UNITED STATES																								
Pringle (subnursery):																								
St. Paul, Minn.-																								
Pathology nursery	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Agronomy nursery	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crookston, Minn.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Morris, Minn.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Golden Valley, Minn.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Duluth, Minn.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Madison, Wis.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Faroo, N. Dak.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brookings, S. Dak.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ames, Iowa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Great Plains (secular):																								
Laurel, N. Dak.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Idahey, N. Dak.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mandan, N. Dak.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dickinson, N. Dak.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Akron, Colo.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CANADA																								
Morden, Manitoba	60	50	70	75	75	75	30	25	30	35	30	80	65	75	75	65	75	80	8	75	75	40	0	0
Winnipeg, Manitoba	40	35	45	40	55	40	4	3	0	10	35	60	90	70	90	90	85	85	3	70	70	50	0	0
Brandon, Manitoba	50	60	15	10	15	10	3	5	1	20	20	50	70	40	50	45	35	40	3	40	40	10	0	0
Indian Head, Saskatchewan	3	2	20	15	10	20	0	0	0	0	20	80	15	10	60	45	10	35	55	60	3	0	0	0
Saskatoon, Saskatchewan	15	10	20	8	25	20	1	1	1	10	10	3	85	30	30	50	70	50	30	40	30	0	0	0
Yorkton, Saskatchewan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Regina, Saskatchewan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Edmonton, Alberta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Calgary, Alberta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Average	48	15	62	22	62	17	3	3	1	27	26	11	41	67	63	65	66	61	2	125	64	7	0	0

a Few but normal pustules.

b Infection not uniform.

c Many plants destroyed by gophers and sparrows.

d Mostly on immature plants.

e Plants dead ripe.

f Destroyed by gophers and sparrows.

g Average of only 23 nurseries.

h Average of only 24 nurseries.

i Average of only 21 nurseries.

TABLE 6.—Percentage of stem rust on 22 varieties of spring wheat and emmer grown in uniform nurseries at 29 experiment stations in the United States in 1921

[T = Trace]

[illegible]

^d Average of only 18 mites/infest.

TABLE 7. Percentage of stem rust on 2½ inches of spring wheat and summer grown uniform nurseries at 49 experiment stations in the United States in 1923

[illegible]

TABLE 8.—Average percentage of stem rust infection on wheat varieties grown in uniform rust nurseries at 34 experiment stations in the United States and Canada during one or more of the five years, 1919 to 1923, inclusive, together with the coefficient of infection, i. e., in comparison with the percentage of infection on Marquis during the corresponding period

Class, variety, and C. I. number	Total number of nursery years	Percentage of stem rust infection					Five-year averages			
		Annual averages					Individual varieties			
		1919	1920	1921	1922	1923	Entire class			
							Weighted average	Marquis during identical period of nursery years	Coefficient of infection (per cent of Marquis)	Weighted average
<i>Durum (Triticum durum)</i>										
Kubanaka	1540	13.5	27.2	17.8	36.7	21.9	23.3	47.6	48.95	19.91
Kubanaka	2094	12.9	27.7	14.6	39.5	20.3	18.7	46.9	39.87	
Kubanaka	4063	25.8	28.1	20.5	37.5	23.9	27.1	47.0	57.66	
Arundaka	1493	63.7	32.8	22.3	38.6	25.5	26.7	46.8	57.05	
Arundaka	4064	29.5	31.8	23.9	38.6	25.5	30.2	46.9	61.38	
Arundaka	6236	17.7	21.8	16.8	35.4	23.4	26.9	47.3	56.86	
Minum	5396	4.8	10.0	3.2	5.6	2.7	4.6	47.1	49.79	
Monad	3284	4.0	9.6	2.8	4.5	2.6	4.4	47.1	9.34	
Monad	3320	1.2	3.4	1.4	4.8	2.0	2.6	47.1	5.48	
Kelba	3322	1.2	3.4	26.6	44.1	32.7	33.8	46.3	73.00	
Kelba	5529	25.5	33.2	28.8	28.9	46.9	61.62	
Peliss	1584	90.94
<i>Common (Triticum vulgare)</i>										
Illynes	2874	66.2	55.0	40.7	57.3	55.1	53.3	47.4	112.45	
Marquis	3641	57.3	41.2	41.0	49.4	51.7	47.4	47.4	100.00	
Marquis	3697	61.7	47.2	37.0	55.2	58.6	50.9	47.6	106.94	
Marquis	6047	38.6	40.8	29.8	42.4	49.4	40.0	47.0	85.10	
Kelb	4800	45.1	48.3	55.7	49.6	46.9	105.75	
Red Bobs	6255	36.0	46.4	54.0	45.1	46.7	96.57	
Red Bobs	2081	65.6	54.3	40.7	61.8	61.2	61.0	47.8	112.97	
Preston	5878	2.8	16.9	2.4	7.8	15.5	8.9	47.4	18.78	
Kora	4523	27.3	39.5	25.5	15.4	38.3	29.1	46.6	62.44	
<i>Club (Triticum compactum)</i>										
Little Club	4066	33.8	53.1	55.6	46.7	46.3	100.86	46.68
<i>Emmer (Triticum dicoccum)</i>										
Emmer	3686	...	1.7	0.4	0.3	0.0	0.8	47.4	1.69	0.51
Khaph	4013	...	0.8	0.0	0.0	0.2	0.3	46.1	0.61	46.90
Average annual epidemic		26.0	27.4	21.9	32.0	30.4				1.09

The varieties which are resistant in the field, however, were less severely rusted than in 1920. The highest percentage of infection on the three most resistant durum varieties, Acme, Monad and Pentad, occurred at Brookings, S. Dak., where from 15 to 18 per cent of infection was recorded. The highest degree of infection on Kota was found in the uniform rust nursery at Fargo, N. Dak., where there was an infection of 35 per cent under conditions of almost complete lodging. In the same nursery the infection on Marquis was as high as 98 per cent. In plot experiments at the same station, where there was no lodging and where the plants were fully mature, Kota had only 10 per cent of stem rust while the infection on Marquis was 80 per cent.

Rust specimens were obtained from each nursery and the material was cultured in the greenhouse at St. Paul for the purpose of identification. The rust responsible for the infection at Chatham, Mich., Madison, Wis., Waseca and Crookston, Minn., Edgeley, N. Dak., Brookings, S. Dak., Lincoln, Nebr., and Akron, Colo., was form 21. At Coon Creek, Minn., and Redfield, S. Dak., forms 21 and 17 were present. From each of the collections made at Duluth and Morris, Minn., and at Ames, Iowa, form 17 alone was isolated. Form 11 was responsible for the epidemic at Langdon, N. Dak. The rust at Fargo, N. Dak., consisted of forms 17 and 9; at St. Paul, Minn., both form 9 and form 32 were found; whereas at Archer, Wyo., form 9 was found in conjunction with form 34, this being the first time form 34 was found in the United States. Two forms were isolated from the Mandan, N. Dak., nursery; *viz.*, form 36 and a form which acted unlike any of those previously described and which was therefore designated as form 38.

It is interesting to note that forms 17, 21, and 34 are absolutely identical with respect to their parasitic behavior on the different varieties grown in the uniform rust nurseries. Form 9 differs from these only by its ability to infect Vernal normally in the greenhouse, which the other forms do not have. Form 11 acts like form 17 but can also attack Kanred (C. I. 5146), while form 17 can not. Form 32 is essentially the same as form 11, except that the infection it produces on the durum is of the "x" type instead of normal. Form 38 behaves in every respect like form 32 except that it can not infect Marquis normally.

Rust Situation in 1923.

In general, the wheat rust infection in the uniform rust nurseries in 1923 agrees fairly closely with the 5-year weighted average, as may be seen from table 8. There was a rather unusually heavy stem rust infection that year on Kota at some of the nurseries in the United States, particularly at Brookings, S. Dak. (60 per cent.); Morris, Minn. (40 per cent.); Fargo,

N. Dak. (35 per cent), etc. In 1920 the percentage of infection on Kota was as high as 50 per cent at Brandon, Manitoba, and 40 per cent at Morden and Winnipeg, Man. It will be interesting to note that the average for Kota in 1923 is almost the same as that in 1920, *i.e.*, 16 and 17 per cent, respectively.

Form 11 was responsible for the rust infection in seven nurseries, *viz.*, St. Paul (pathology and agronomy nurseries), Crookston, Fargo, Ames, Langdon and Archer; form 21 was dominant in six nurseries, namely, Morris, Coon Creek (sandy soil), Brookings, Lincoln, Edgeley and Akron; form 32 predominated in the following five nurseries: Coon Creek (peat bog), Duluth, Chatham, Dickinson, and Redfield; form 29 appeared in two nurseries, Waseca and Madison; whereas form 17 was obtained only from Mandan. The rust estimates for each variety at each nursery are given in table 7.

From the results presented in tables 3 to 7 inclusive, and from the accompanying discussions it can be seen that some varieties rusted heavily at most of the stations each year. Nearly all the hard red spring wheat varieties grown were susceptible to the prevailing physiologic forms. Some varieties of durum were considerably more susceptible than others; but, in general, as shown in table 8, all of the durum varieties as a class had a lower percentage of infection than either Marquis or the entire class of common wheats (approximately 20 per cent for the durums as against 43 per cent \pm for the common varieties). Pentad, Monad, and Acme in the order named were the most resistant durum varieties under field conditions. Kota was much more resistant to stem rust attack during five years as a whole than was Marquis, the data showing about 9 per cent for the former, as compared with over 47 per cent for the latter. Marquis escaped stem rust infection slightly better than either Kitchener, Power Fife, Haynes Bluestem, or Preston, in the order named. However, both Prelude and Ruby fared better than Marquis. The two varieties of spring emmer (Vernal and Khapli) were virtually immune from stem rust in the field, although, as recorded in table 11, during the 5-year period, two physiologic forms which infected Vernal very readily under greenhouse conditions (forms 9 and 30) were isolated 11 different times. Little Club was completely susceptible throughout.

Greenhouse and Laboratory Studies of Physiologic Forms

Rust specimens were collected each year at some or all of the uniform rust nurseries and sent to St. Paul for identification. During the period under review 78 collections were cultured in the greenhouses at University Farm and the identity of the physiologic forms isolated was established.

TABLE 9.—Occurrence of physiologic forms of *Puccinia graminis tritici* during the 5 years, 1919–1923, inclusive, at different uniform rust nurseries in the United States and Canada

Experiment stations	Physiologic forms found in:					Number of forms isolated from each station
	1919	1920	1921	1922	1923	
<i>United States</i>						
Akron, Colo.	17.....	21.....	21.....	2
Ames, Iowa	3.....	30.....	17.....	11.....	4
Archer, Wyo.	11.....	9, 31.....	11.....	3
Brookings, S. Dak.	3, 5.....	17.....	21.....	21.....	4
Chatham, Mich.	21.....	32.....	2
Coon Creek, Minn.	17, 21.....	21, 32.....	3
Crookston, Minn.	9.....	21.....	11.....	3
Dickinson, N. Dak.	9.....	32.....	2
Dubuque, Minn.	21.....	17.....	32.....	3
Edgeley, N. Dak.	9, 37.....	21.....	21.....	3
Fargo, N. Dak.	3, 22.....	21, 29.....	9, 17.....	11.....	7
Langdon, N. Dak.	21.....	11.....	11.....	2
Lincoln, Nebr.	13.....	11, 13.....	21.....	21.....	3
Madison, Wis.	37.....	21.....	29.....	3
Mandan, N. Dak.	17.....	36, 38.....	17.....	3
Morris, Minn.	17.....	21.....	2
Redfield, S. Dak.	17, 21.....	32.....	3
St. Paul, Minn.	3, 12.....	3a.....	1, 17, 18, 29.....	9, 32.....	11b.....	11
Waseca, Minn.	30, 36.....	21.....	29.....	2
<i>Canada</i>						
Brandon, Man.	3, 29.....	3
Edmonton, Alta.	29.....	9, 17.....	3
Indian Head, Sask.	12, 17.....	29.....	3
Morden, Man.	3, 32.....	29.....	3
Rosethorn, Sask.	3, 9, 17.....	29, 32.....	5
Saskatoon, Sask.	17.....	24.....	2
Winnipeg, Man.	17.....	17, 32.....	2
Most prevalent forms in any one year, in their order of prevalence.						
	3, 17.....	17, 3, 29....	29, 9, 17, 21	21, 17, 9....	11, 21, 32....	

a Nursery inoculated with several physiologic forms, but form 3 was seemingly dominating.

b Culture badly mixed, but form 11 apparently predominated.

Identity and Nature of Physiologic Forms Isolated.

The identity of the rusts collected was determined by the method described by Stakman and Levine (30). The various types of infection produced by different physiologic forms of *P. graminis tritici* on seedling plants are illustrated in Plate I. The forms isolated from a given nursery in a given year are listed in table 9. Most of the collections made, namely, 57, consisted of a single form each; 20 contained two forms each; while three different forms were isolated from one individual collection. The classes of host reaction and the corresponding types of rust infection, resulting from inoculation of seedling wheat plants with spores of *Puccinia graminis tritici*, are described below.

Different types of infection produced by physiologic forms of *Puccinia graminis tritici* on seedlings of different varieties of wheat.

CLASS "R" (Varieties resistant) is indicated by infection types, 0, 1, and 2, and their plus and minus fluctuations.

Type (0)—Host practically immune—No uredinia are developed, but sharply defined hypersensitive flecks or necrotic lesions are usually present;

Type (1)—Host extremely resistant.—Infection very light; uredinia minute and scattered and surrounded by very sharply defined, continuous necrotic areas;

Type (2)—Host moderately resistant.—Infection light; uredinia isolated and small to medium in size; hypersensitive areas in the form of necrotic halos or circles; pustules usually in green, but slightly chlorotic, islands.

CLASS "S" (Varieties susceptible) is indicated by infection types 3 and 4, and their plus and minus fluctuations.

Type (3)—Host relatively susceptible.—Infection moderate; uredinia midsized with slight tendency to coalesce; true hypersensitiveness absent, but light chlorotic areas usually present, especially under unfavorable cultural conditions.

Type (4)—Host completely susceptible.—Infection normal and heavy; uredinia large and generally confluent; hypersensitiveness normally absent, but chlorosis may be present when cultural conditions are unfavorable.

CLASS "I" (Reaction indeterminate) is indicated by infection type X with the accompanying plus and minus fluctuations.

Type (X)—Host intermediately susceptible.—Infection of a heterogeneous nature; uredinia very variable, apparently in-

cluding all types and degrees of infection, often on the same blade; no mechanical separation seems to be possible, since, on reinoculation, spores from small uredinia may produce large ones, and *vice versa*. In general, the infection is ill defined.

The nature of the parasitic behavior of the different physiologic forms isolated is shown in table 10. It will be seen from this table that all these forms, except form 38, which was found together with form 36 at Mandan, N. Dak., in 1922, attack Marquis very readily. It will be interesting to note, also, that in the greenhouse Kota is more or less susceptible to all of these forms except form 24, which was found at Saskatoon, Sask., in 1921. All of the hard red spring varieties tested in the nurseries, except Kota, reacted like Marquis to the different forms isolated, *i. e.*, they were susceptible to them and might therefore be considered as a group unit. The durumms are different in this respect because they all reacted differently to

TABLE 10.—*Reaction of different varieties of wheat to physiologic forms of stem rust isolated from different uniform rust nurseries in the United States and Canada during the 5 years, 1919-1923, inclusive*

Physiologic forms isolated	Mean reactions ^a of differential varieties											
	Club	Hard Red				Durum				Eln- korn	Emmer	
	Little Club C. I. 4066	Marquis C. I. 3641	Kamek C. I. 5146	Kota C. I. 5878	Amnauka C. I. 4072	Mandan C. I. 5296	Amnauka C. I. 6236	Kubanka C. I. 2091	Acme C. I. 5281	Elnkorn C. I. 2483	Vernal C. I. 3686	Klappi C. I. 4013
1.....	4	4	0	3	1	1	1	3	3++	3	0	1
3.....	4	4	4	3	1	1	1	1	3	3	1	0
5.....	4	4	0	3	4	3	3	1	3	3	0	0
9.....	4	4	0	3	4	4	4	4	3	3+	4	1
11.....	4	4	3	3	4	4	4	3	3	3	1	1
12.....	4	4	4	3	4	1	1	1	3	3	1	0
13.....	4	4	3	3	4	3	3	2	3	3	1	1
17.....	4	4	0	3	4	4	4	3	3	3	1	1
18.....	4	4	4	3	1	1	1	3	3	3	1	1
21.....	4	4	0	3	4	4	4	4	3	1	0	1
22.....	4	4	4	3	1	4	4	0	3	3	1	0
24.....	4	4	0	2	4	4	4	3	3	3	1	0
29.....	4	4	0	3	X++	X	X	X	X	3	1	1
30.....	4	4	0	3	X++	X	X	X	X	3+	4	1
32.....	4	4	4	3	X	X	X	X	X	3	1	1
34.....	4	4	4	4	4	4	4	4	3	1	0	1
36.....	4	4	4	3	1	1	0	X	3	3	0	1
37.....	4	4	0	3	4	4	4	X	3	3	1	1
38.....	4	2	4	3	X	X	X	X	X	4	1	1

^a *Explanation of symbols.* 0—Absolute immunity; 1—Extreme resistance; 2—Moderate resistance; 3—Moderate susceptibility; 4—Complete susceptibility; X—Heterogeneous reaction; (:)—Hypersensitive fleck; (.)—Necrotic lesions. Plus and minus signs indicate a slightly greater or less amount of rust than the nearest figure representing the infection type (30).

certain individual forms. Vernal emmer was susceptible in the greenhouse to two of the forms isolated, *viz.*, forms 9 and 30. Khapli and Little Club reacted uniformly to all of the physiologic forms, the former being extremely resistant and the latter completely susceptible to all of them.

Some of the forms isolated are quite virulent and others are very weak. Under favorable conditions, forms 11, 17, and 21 can attack very heavily all of the varieties tested in the uniform nurseries except Vernal and Khapli emmers. These forms are not identical, however, because they vary greatly in their parasitism on other varieties. They can be separated from each other on the basis of their action on Kanred (C. I. 5146) and Einkorn (C. I. 2433).

Forms 9 and 30 can infect Vernal (C. I. 3686) in the greenhouse. Form 30 differs from form 9 only in that its action on the durum is heterogeneous and it is therefore somewhat less virulent than form 9. A similar relationship exists between forms 32 and 11 and forms 29 and 17. Marquis is resistant to form 38, but in all other respects this form behaves just like form 32. Form 37 approaches form 17 more closely than does form 29, there being just one point of difference between the first two, namely, Kubanka (C. I. 2094) alone reacting indeterminately to form 37, whereas all the durum differentials react thus to form 29.

Forms 13, 22, 12, and 18 bear a certain relation to form 11. But form 13 differs from 11 in not being able to attack Kubanka (C. I. 2094) normally. Form 22 is one degree less virulent than form 13, Arnautka (C. I. 4072) and also Arnautka (C. I. 4064 and C. I. 1493) being resistant to it in addition to Kubanka (C. I. 2094). Form 12, although capable of producing heavy infection on Arnautka (C. I. 4072), can infect Arnautka (C. I. 6236), Mindum (C. I. 5296), and Kubanka (C. I. 2094) only with great difficulty. This order is slightly shifted in the case of form 18, to which both Arnautka strains, as well as Mindum, are highly resistant, but Kubanka is very susceptible. Form 36 is slightly less virulent than form 18, as it produces a heterogeneous type of infection on Kubanka (C. I. 2094). This variety is completely resistant to form 3, otherwise form 3 is identical with forms 36 and 18.

Form 5 is similar to form 17 and differs from form 17 only in that it can not normally infect Kubanka (C. I. 2094). Form 1, like form 17, is capable of producing heavy infection on Kubanka, but Mindum and the two strains of Arnautka are extremely resistant to it. In this respect it differs from form 17. Form 24 also is closely allied to form 17 and differs from it in just one particular: it can not attack Kota normally either in the field or greenhouse.

Prevalence and Distribution of Physiologic Forms Isolated

From 1919 to 1923, inclusive, 19 different forms of *P. graminis tritici* were isolated from the nurseries. It will be seen from table 11 and figure 3

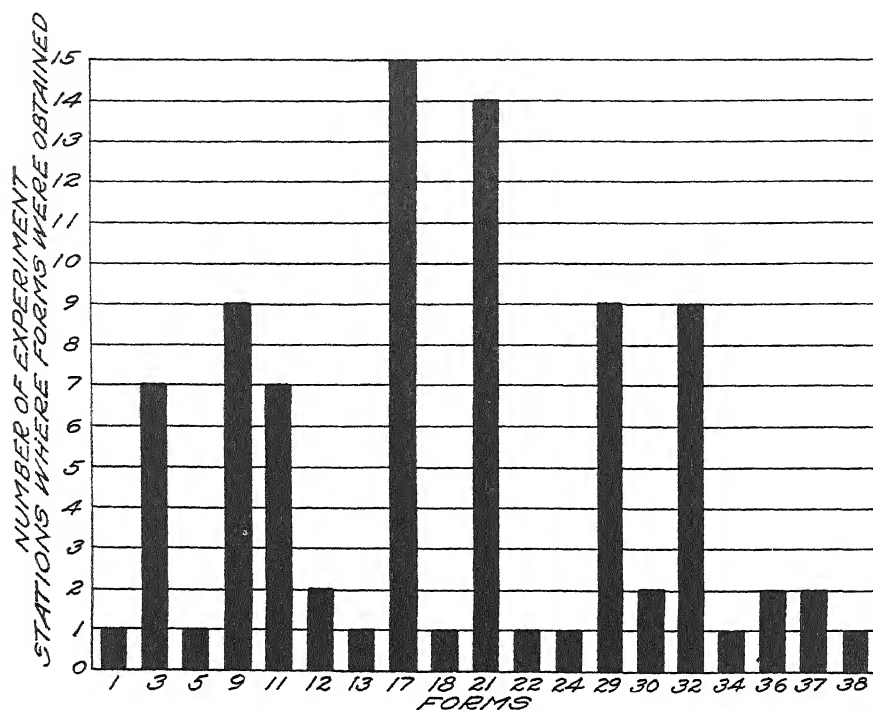


FIG. 3. Prevalence of physiologic forms of *Puccinia graminis tritici* isolated from uniform rust nurseries during the 5-year period, 1919-1923, inclusive.

that form 17 was isolated most frequently and from the greatest number of localities. Margaret Newton (20) found this form to be quite widely distributed also in Canada. It appeared to a greater or less extent in the uniform rust nurseries each year and was isolated from 15 different localities. Table 9 shows that form 17 occupied the second place in frequency of occurrence in 1919, form 3 being first. However, form 17 led the frequency list in 1920, but in 1921 it again dropped to the second place, which it shared with form 9. Form 29 headed the frequency list in 1921, was not found in the nurseries in 1922 and was at the bottom of the list in 1923. Form 21 was found in 14 different nurseries, but apparently was a factor in the rust epidemic only during the last three years of the experiment. In 1921 it was isolated from three localities, holding the third frequency position; in 1922 it appeared in 10 different places of the experimental area and was the first on the list; in 1923 it shared this position with form 11, having been found at six of the stations.

Forms 11 and 32 both were isolated in four different years, but during this period they were found only at seven and nine separate nurseries,

respectively. Forms 9 and 29 were found three different years, the former in 1920, 1921, and 1922; the latter in 1920, 1921, and 1923. Both appeared in nine different localities. Forms 3, 12, and 36 each were collected for two consecutive years, the first two forms in 1919 and 1920, the last in 1921 and 1922. Form 3 was found at seven different localities, whereas forms 12 and 36 each were found at only two localities. The remaining forms occurred only in individual years, and each of them, except forms 30 and 37, in only a single locality. Form 30 and form 37 both were found in 1921 and in two different nurseries, the first at Ames, Iowa, and St. Paul, Minn., the last at Edgeley, N. Dak., and Madison, Wis.

Too sweeping conclusions should not be drawn from the above discussion on distribution and prevalence of physiologic forms. It must be remembered that, after all, only a rather limited number of collections were made at these stations, yielding a comparatively small number of individual cultures. It should also be borne in mind that collections were not made each year from every nursery. Nevertheless, there seems to be fair evidence that certain forms occur more frequently than others and that some forms have a wider range of distribution than other forms. Sometimes the most frequently occurring forms are also the most widely distributed ones, and *vice versa*; but this is not necessarily always the case. Neither the prevalence of a physiologic form, nor its distribution, could be correlated with the degree of its virulence on the differential hosts.

Several factors affect the prevalence and distribution of physiologic forms of *P. graminis tritici*. It has been demonstrated (11, 38, 36, 15) that the parasitism of the different varieties of *P. graminis* is profoundly affected by resistant hosts. It is quite obvious, therefore, that resistant varieties of wheat, grown in a given region, impose a definite restraint on the occurrence of certain forms of *P. graminis tritici* in that region. It also has been shown (13, 6, 23) that the physiologic forms of stem rust differ: first, in the germinability of their spores in diverse temperature ranges; second, in their ability to develop in the host plant under different environmental conditions; and, third, in their capacity to hibernate in the host tissue. Consequently, it is very likely that the difference in the climatic conditions in the various parts of the country plays no small part in the distribution of these forms. In addition to this, there also are the prevailing winds and the upper air currents, and possibly the fortuitous mutational origin of physiologic forms, which must be taken into consideration. Then there also is the possibility that the different stem rust forms originate through hybridization in the aecial stage (33). In this case the common barberry would be another important factor influencing their distribution and prevalence. While the presence of some of these factors and the absence of others may not be sufficient to prevent the appearance of

TABLE 11.—Prevalence during the 5 years, 1919–1923, inclusive, at various uniform rust nurseries in the United States and Canada of different physiologic forms of *Puccinia graminis tritici*

Physiologic forms isolated	Stations from which physiologic forms were isolated in:					Number of localities at which each physiologic form was found during 5 year period
	1919	1920	1921	1922	1923	
1	Ames, Iowa Brookings, S. Dak. Fargo, N. Dak. St. Paul, Minn.	St. Paul, Minn. Brandon, Man. Morden, N. Dak. Rosthern, Sask.	St. Paul, Minn.			1
3	Brookings, S. Dak.					7
5						1
9		Rosthern, Sask.	Cookston, Minn. Edgeley, N. Dak. Brandon, Man. Edmonton, Alta.	Archer, Wyo. Dickinson, N. Dak. Fargo, N. Dak. St. Paul, Minn.		9
11	Archer, Wyo.	Lincoln, Nebr.		Langdon, N. Dak.	Ames, Iowa Archer, Wyo. Cookston, Minn. Fargo, N. Dak. Langdon, N. Dak. St. Paul, Minn.	7
12	St. Paul, Minn.	Indian Head, Sask.				2
13	Lincoln, Nebr.	Lincoln, Nebr.				1
17	Akron, Colo. Mandan, N. Dak.	Mandan, N. Dak. Indian Head, Sask. Rosthern, Sask. Saskatoon, Sask. Winnipeg, Man.	Brookings, S. Dak. St. Paul, Minn. Edmonton, Alta. Winnipeg, Man.	Ames, Iowa Coon Creek, Minn. Duluth, Minn. Fargo, N. Dak. Morris, Minn. Redfield, S. Dak.	Mandan, N. Dak.	15
18			St. Paul, Minn.			1
21			Duluth, Minn. Fargo, N. Dak. Langdon, N. Dak.	Akron, Colo. Brookings, S. Dak. Cuthbert, Mich. Coon Creek, Minn. Cookston, Minn. Edgeley, N. Dak. Lincoln, Nebr. Maudslon, Wks. Redfield, S. Dak. Waseca, Minn.	Akron, Colo. Brookings, S. Dak. Coon Creek, Minn. Edgeley, N. Dak. Lincoln, Nebr. Morris, Minn.	14

TABLE 11.—(Continued)

Physiologic forms isolated	Stations from which physiologic forms were isolated in:					Number of localities at which each physiologic form was found during 5-year period
	1919	1920	1921	1922	1923	
22	Fargo, N. Dak.					1
24			Saskatoon, Sask.			1
29		Brandon, Man. Edmonton, Alta.	Fargo, N. Dak. St. Paul, Minn. Brandon, Man. Indian Head, Sask. Morden, Man. Rosthern, Sask.		Madison, Wis. Waseca, Minn.	9
30			Ames, Iowa St. Paul, Minn.			2
32		Morden, Man.	Rosthern, Sask. Winnipeg, Man.	St. Paul, Minn.	Clutham, Mich. Coon Creek, Minn. Dickinson, N. Dak. Duluth, Minn. Redfield, S. Dak.	9
34				Archer, Wyo.		1
36			St. Paul, Minn.	Mandan, N. Dak.		2
37			Edgeley, N. Dak. Madison, Wis.	Mandan, N. Dak.		2
38						1
Number of physiologic forms isolated during any one year.	7	8	11	8	5	

the physiologic forms of *P. graminis tritici*, they undoubtedly play a notable rôle in determining the relative prevalence of these forms and the extent of their distribution.

Morphology of Some Important Physiologic Forms

It is of course impossible to distinguish varieties and physiologic forms of *P. graminis* by macroscopic or superficial microscopic examination, although the group forms or varieties can be distinguished from each other by careful measurements of spores.

The study of the comparative morphology of several physiologic forms of *P. graminis tritici* was undertaken to ascertain whether they possessed morphological differences as do the group-forms, or varieties, of *P. graminis* (29, 15, 31). Eight of the more important forms, two of which (3 and 27) consisted of duplicate strains, were chosen for this study. The history and behavior of each form are given in tables 12 and 13.

Form 1 was selected because it is the oldest physiologic form of *P. graminis tritici* now available, having been cultured continuously for more than nine years. It readily attacks half of the 12 differential hosts. The resistant varieties are: Kanred (C. I. 5146) in the *vulgare* group; Arnautka (C. I. 4072), Mindum (C. I. 5296), and Arnautka-“Speltz Marz” (C. I. 6236) among the durumms; and the two emmer varieties, Vernal (C. I. 3686) and Khapli (C. I. 4013).

Form 3 is one of the fairly prevalent forms and was chosen because it differs from form 1 in only two respects: Kanred (C. I. 5146) is entirely immune from form 1 but completely susceptible to form 3; while the contrary is true of Kubanka (C. I. 2094), which is highly susceptible to the first but very resistant to the latter form.

Form 9 is one of the most virulent physiologic forms and rather common in occurrence. It produces heavy infection on all the differential hosts with the exception of Kanred and Khapli.

Form 15 is rare, but it is the most virulent of all the forms so far known, as every one of the differentials, except Khapli, is completely susceptible to it.

Form 17 appears to be the most widely distributed and the most prevalent. It also is quite virulent, being able to infect normally all of the differential hosts except Kanred, Vernal emmer, and Khapli emmer.

Form 27, thus far, has been found only once on the American Continent but has been collected in Asia and in several European countries. Parasitically it is one of the weaker forms, infecting heavily only four of the 12 differential hosts, viz., Little Club (C. I. 4066), Kubanka (C. I. 2094), Acme (C. I. 5284), and Vernal emmer (C. I. 3686). Among the varieties resis-

TABLE 12.—History of physiologic forms of *Puccinia graminis tritici* studied morphologically

Physiologic form	Original host	Place of collection	Date collected	Name of collector	Ultimate host	Culture
1	Wheat	St. Paul, Minn.	Sept. 12, 1915	E. C. Stakman and F. J. Pfenkel	Little Club	Monosporous
3a	do	Stillwater, Okla.	Oct. 18, 1917	E. C. Stakman and Chas. Drechsler	do	Multisporous
3b	do	Lehi, Utah	Aug. 30, 1923	R. U. Crotter	do	Monosporous
9	Barley	De Pere, Wis.	July 27, 1918	R. E. Vaughan	do	do
15	Wheat	Pusa, India	March 12, 1923	R. R. Sen	Vernal	Multisporous
17	Agropyron	Boise, Okla.	Sept. 13, 1923	E. A. Langren	Little Club	Monosporous
27a	Wheat	Pusa, India	Feb. 26, 1923	R. R. Sen	Vernal	Multisporous
27b	do	Berkeley, Calif.	March 19, 1924	W. W. Mackie	do	do
29	Barley	Huntley, Ill.	June 23, 1922	J. L. Seal	Marquis	do
38	Wheat	Mandan, N. Dak.	Aug. 10, 1922	M. N. Levine	Little Club	do

tant to this form are: Marquis (C. I. 3641), Kota (C. I. 5878), and Einkorn (C. I. 2433), all of which are very susceptible to the five forms enumerated above.

Form 29 is interesting mainly on account of the heterogeneous type of infection it produces on the durum varieties. It occurs rather frequently and is fairly widespread in its distribution.

Form 38 was first isolated in 1922 but has been found several times since then. The reaction of the durum varieties to this form, as to form 29, is indeterminate. The chief difference in the pathogenicity of these two forms appears on two hosts, Marquis and Kanred. Marquis is susceptible to form 29 and resistant to form 38, whereas the opposite is the case with Kanred.

TABLE 13.—*Behavior of physiologic forms of Puccinia graminis tritici studied morphologically, as shown by type of infection produced on differential hosts*

Mean reaction of differential varieties of wheat and wheat allies												
	Club	Hard	Red	Common	Durum					Ein-korn	Emmer	
Physiologic forms	Little Club C. I. 4066	Marquis C. I. 3641	Kanred C. I. 5146	Kota C. I. 5878	Arnakka C. I. 4072	Mhudum C. I. 5296	Arnakka C. I. 6236	Kubanka C. I. 2094	Aene C. I. 5284	Einkorn C. I. 2433	Vernal C. I. 3686	Khapli C. I. 4013
1.....	4	4 -	0	3 +	1 =	1	1 =	3 +	3 + +	3	0;	1 =
3.....	4	4 -	4 =	3 +	1 =	1 =	1 =	1 +	3 + +	3 +	1 =	0;
9.....	4	4 -	0	3 + +	4 -	4 =	4 =	4 =	3 + +	3 +	4 =	1 -
15.....	4	4 -	4 =	3 + +	4 =	4 =	4 =	3 + +	3 + +	3 +	4 =	1 =
17.....	4	4 -	0.	3 +	1 =	4 =	4 =	3 + +	3 + +	3	1 =	1 =
27.....	4 =	4	0.	0.	1 =	1	1 -	4 =	3 + +	1 =	4 =	1 +
29.....	4	4 -	0	3	X 1 +	X	X +	X	X + +	3	1 -	1 -
38.....	4	4 =	4 -	3 + +	X +	X =	X +	X + +	X + +	4 -	1 =	1 +

In the previous studies it was found that 100 spore measurements gave equally as good results as 200 or 400, whereas when less than 100 spores were measured the results were not always representative or conclusive. Inasmuch as the work in the past dealt with varieties, it was not certain that the earlier findings would hold good for the physiologic forms within these varieties. In order to avoid errors which might result from measuring an insufficient number of spores, a large and fully developed uredinium of the monosporous culture of *P. graminis tritici*, form 1 Stak. and Lvne. was selected for the purpose of determining what constitutes an adequate, representative, random sample in the present investigation.

Four hundred spores of the uredinium chosen were measured at one time, and the length and width of each recorded separately. Comparisons

were then made between independent samples of the same and different magnitudes, and also of sub-samples as contrasted with general or composite samples. The probable and standard errors of the differences in the arithmetical means of the values compared were determined by the formulae suggested for the different comparisons by Pearson (22) and Yule (41).

In the case of independent samples, the test of significant and non-significant differences of type was made by comparing the difference of the means ($m_1 - m_2$) with the probable error of this difference, according to the generally accepted formula: $m_1 - m_2 \div 0.6745 \sqrt{\sigma_1^2/n_1 + \sigma_2^2/n_2}$; where m_1 , n_1 , σ_1 and m_2 , n_2 , σ_2 are the respective means, magnitudes and standard deviations of any two independent samples compared.

On the supposition that a quantity is significant when it is β times its standard deviation, or $\beta/0.67449$ times its probable error, Pearson (22) advises the following formula for the significance test of the difference of types in a sub-sample (n , m , σ) and general sample (N , M , Σ), of which the sub-sample is a component part, viz;

$$m - M > \beta \sqrt{\frac{\sigma^2}{n} - \frac{2\sigma^2 - \Sigma^2}{N} - \frac{n(M - m)^2}{N(N - n)}}$$

this being true whatever the magnitude of N and n . Pearson (22) states that "using the above formula it may be that a considerable number of cases, for which no proof of significant differentiation has been given, and which have been taken accordingly as having no differentiation, can now be demonstrated to have significant differentiation."

Sub-samples were abstracted, combined, and compared with respective general samples, because it was desired to ascertain how the enlargement of a smaller sample by continuously adding on of more individuals would affect the frequency distribution and the constancy of variates. But, as a sub-sample is made larger and larger, the value of its mean approaches closer and closer to that of the general sample and thus the probable error of the difference becomes less and less and ultimately vanishes. However, by the use of the first formula, it approaches the finite value $0.67449 \sqrt{2\Sigma^2/N}$. For this reason the second formula was used in the attempt to determine the possible existence of significant differences between sub-samples and general samples of different magnitudes, though of the same source.

The results obtained are recorded in tables 14 to 17, inclusive. Figures 4 to 9 give a graphic picture of the relative size and shape of the uredinospores in the different samples. The curves and the bars are plotted vertically on a logarithmic scale, because it shows the proportional as well as the actual dispersion of the variates in samples of unequal size, and it also enables one to make a direct comparison of rates of change.

TABLE 14.—Variations and constants for urediniospore lengths of different random samples procured from a single sorus of a monosporous culture of *Puccinia graminis tritici* form 1

Lot no.	Random samples studied		Spore classes according to length										Size limits	Constants	
	Type	Magnitude	21 μ	24 μ	27 μ	30 μ	33 μ	36 μ	39 μ	42 μ	Mean length	Standard deviation		Coefficient of variability	
1	Independent	50		1	3	10	14	16	6		23.46—40.25	33.30 \pm 0.31	3.21 \pm 0.22	9.61 \pm 0.65	
2		100			2	20	36	33	7	2	25.53—41.17	33.87 \pm 0.20	2.98 \pm 0.11	8.80 \pm 0.42	
3		200			1	4	12	29	70	58	21	5	21.16—43.24	33.69 \pm 0.18	3.71 \pm 0.13
4	Composite	50			2	12	9	23	3	1	25.53—40.71	33.96 \pm 0.31	3.26 \pm 0.22	9.60 \pm 0.65	
5		100		1	5	22	23	39	9	1	23.46—40.71	33.75 \pm 0.23	3.40 \pm 0.16	10.07 \pm 0.48	
6		200			1	7	42	59	72	16	3	23.46—41.17	33.81 \pm 0.15	3.20 \pm 0.11	9.40 \pm 0.32
7		300		1	4	18	59	96	96	22	4	21.16—41.86	33.41 \pm 0.13	3.45 \pm 0.10	10.32 \pm 0.28
8		400		1	5	19	71	129	130	37	8	21.16—43.24	33.75 \pm 0.11	3.46 \pm 0.08	10.25 \pm 0.24

TABLE 15.—Variations and constants for urediniospore widths of different random samples procured from a single sown of a monosporous culture of *Puccinia graminis tritici* form 1

Lot no.	Random samples studied		Spore classes according to width										Size limits	Constants		
	Type	Magnitude	16 μ	17 μ	18 μ	19 μ	20 μ	21 μ	22 μ	23 μ	24 μ	Mean width		Standard deviation	Coefficient of variability	
1	Independent	50			5	3	25	13	3	1		18.17—23.46	20.18 \pm 0.10	1.05 \pm 0.07	5.20 \pm 0.35	
2		100	1	2	4	12	45	23	9	4		16.33—23.23	20.23 \pm 0.08	1.23 \pm 0.06	6.08 \pm 0.30	
3		200	1	1	12	38	82	39	20	6	1	16.79—24.61	20.32 \pm 0.06	1.18 \pm 0.04	5.81 \pm 0.20	
4	Composite	50		1	4	11	19	10	5			17.25—22.54	19.96 \pm 0.11	1.15 \pm 0.08	5.76 \pm 0.39	
5		100		1	9	14	44	23	8	1		17.25—23.46	20.07 \pm 0.07	1.11 \pm 0.05	5.53 \pm 0.26	
6		200	1	3	13	26	89	46	17	5		16.33—23.46	20.15 \pm 0.06	1.17 \pm 0.04	5.81 \pm 0.20	
7		300	2	4	19	45	125	67	31	7		16.33—23.46	20.16 \pm 0.05	1.20 \pm 0.03	5.95 \pm 0.16	
8		400	2	4	25	61	171	85	37	11	1	16.33—24.61	20.16 \pm 0.04	1.19 \pm 0.03	5.90 \pm 0.14	

TABLE 16.—Variations and constants for urediniospore shapes of different random samples procured from a single sorus of a monosporous culture of *Puccinia graminis tritici* form 1

Lot no.	Random samples studied		Spore classes according to ratio of length to width										Correlation coefficients	Constants		Coefficient of variability
	Type	Magnitude	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	Mean ratio	Standard deviation				
1	Independent	50		1	9	16	18	3	3		1.788±0.021	0.220±0.015	12.30±0.83			
2		100		1	13	39	30	13	3	1	1.708±0.014	0.212±0.010	12.41±0.59			
3		200	2	11	18	71	50	40	5	3	1.711±0.012	0.251±0.009	14.85±0.50			
4	Composite	50		1	7	17	19	3	3		1.700±0.020	0.213±0.014	12.53±0.85			
5		100		2	16	33	28	15	6		1.712±0.016	0.232±0.011	13.56±0.65			
6		200		3	30	72	58	28	9		1.705±0.010	0.218±0.007	12.79±0.35			
7		300	1	12	41	109	83	12	10	2	1.691±0.009	0.233±0.006	13.78±0.38			
8		400	2	14	18	111	109	68	14	4	1.711±0.008	0.230±0.006	13.97±0.33			

TABLE 17.—Summary of differences between the means of urediniospore dimensions of different random samples procured from a single sorus of a monosporous culture of *Puccinia graminis tritici* form 1

Type of samples	Random samples compared	LENGTH		WIDTH		THICKNESS	
		Difference in means (in microns)	Difference divided by P. E.	Difference in means (in microns)	Difference divided by P. E.	Difference in means (in microns)	Difference divided by P. E.
INDEPENDENT	50 spores and 50 spores	0.66±0.44	1.50	0.22±0.15	1.47	0.088±0.029	3.03
	do.....100 do	0.57±0.37	1.54	0.05±0.13	0.38	0.080±0.025	3.20
	do.....200 do	0.39±0.36	1.08	0.14±0.12	1.16	0.077±0.024	3.21
	100 spores and 100 spores	0.12±0.30	0.40	0.16±0.11	1.45	0.004±0.021	0.19
	do.....200 do	0.18±0.27	0.67	0.09±0.10	0.90	0.003±0.018	0.67
	200 spores and 200 spores	0.12±0.23	0.52	0.17±0.08	2.13	0.006±0.016	0.38
	50 spores and 100 spores	0.21±0.33	0.64	0.11±0.11	1.00	0.012±0.022	0.55
	do.....200 do	0.15±0.40	0.38	0.19±0.14	1.36	0.005±0.026	0.19
	do.....300 do	0.55±0.43	1.28	0.20±0.15	1.33	0.000±0.028	0.32
	do.....400 do	0.21±0.41	0.48	0.20±0.15	1.33	0.011±0.029	0.38
COMPOSITE	100 spores and 200 spores	0.06±0.22	0.27	0.08±0.68	1.00	0.007±0.015	0.47
	do.....300 do	0.34±0.28	1.21	0.09±0.09	1.00	0.021±0.019	1.11
	do.....400 do	0.00±0.30	0.00	0.09±0.10	0.90	0.001±0.020	0.05
	200 spores and 300 spores	0.40±0.15	2.67	0.01±0.04	0.25	0.014±0.010	1.40
	do.....400 do	0.06±0.17	0.35	0.01±0.06	0.17	0.006±0.012	0.50
	300 spores and 400 spores	0.34±0.10	3.40	0.00±0.03	0.00	0.020±0.007	2.86

The individual spore lots Nos. 1, 2, and 3, and the pair lots 1 and 4, 2 and 5, and 3 and 6 are independent samples. Lot No. 5 is composed of lots 1 and 4; and it in turn is a sub-sample of lot No. 6. Lots 5 and 6 together constitute lot No. 7. All of the sub-samples, of course, enter into the make-up of lot No. 8.

As may be observed from table 17, there seems to be no significant difference between the means of independent samples of 50, 100, and 200 urediniospores procured from the same culture and studied under the same conditions. Likewise, the differences in the size and shape of these spores in composite samples of 50, 100, 200, 300, and 400 measurements may be considered, almost without exception, as quite insignificant statistically. There also was very little difference in the variability of the different lots, in no case much higher than two per cent (See tables 14-16 and figures 4-9).

The probable errors of the means, as might be expected, were consistently and gradually smaller as the sizes of the population were increased. The reduction was in close agreement with the mathematical expectation (19, 41). The contour of the frequency curves in the larger samples, as illustrated in figures 4 and 5 and 7 and 8, approached more nearly that of the "normal" curve. There was no perceptible difference in the mean ratio of the long to the short diameters of the urediniospores in the different

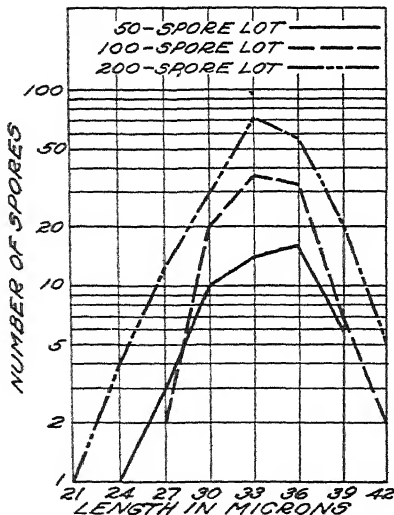


FIG. 4

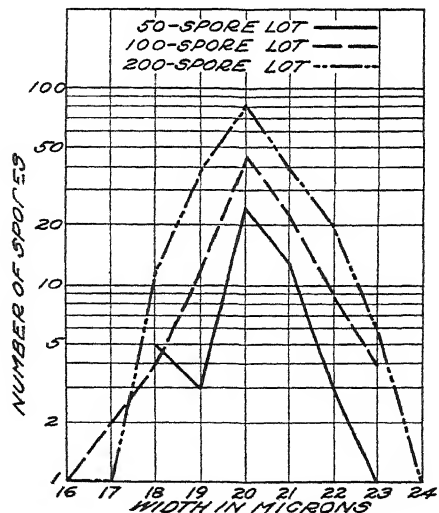


FIG. 5

FIG. 4. Similarity in length of urediniospores of independent random samples of different magnitude obtained from a single sorus of *Puccinia graminis tritici* form 1.

FIG. 5. Similarity in width of urediniospores of independent random samples of different magnitude obtained from a single sorus of *Puccinia graminis tritici* form 1.

lots, with the possible exception of the 50-spore lot No. 1, when compared with the larger independent samples, Nos. 2 and 3.

From the data presented above it would appear that random samples of even 50 spores perhaps might be sufficiently large for a study of the comparative morphology of physiologic forms of *P. graminis tritici*. But because of the relative irregularity in the frequency distribution in the 50-spore lots, it was decided to consider samples of not less than 100 spores as

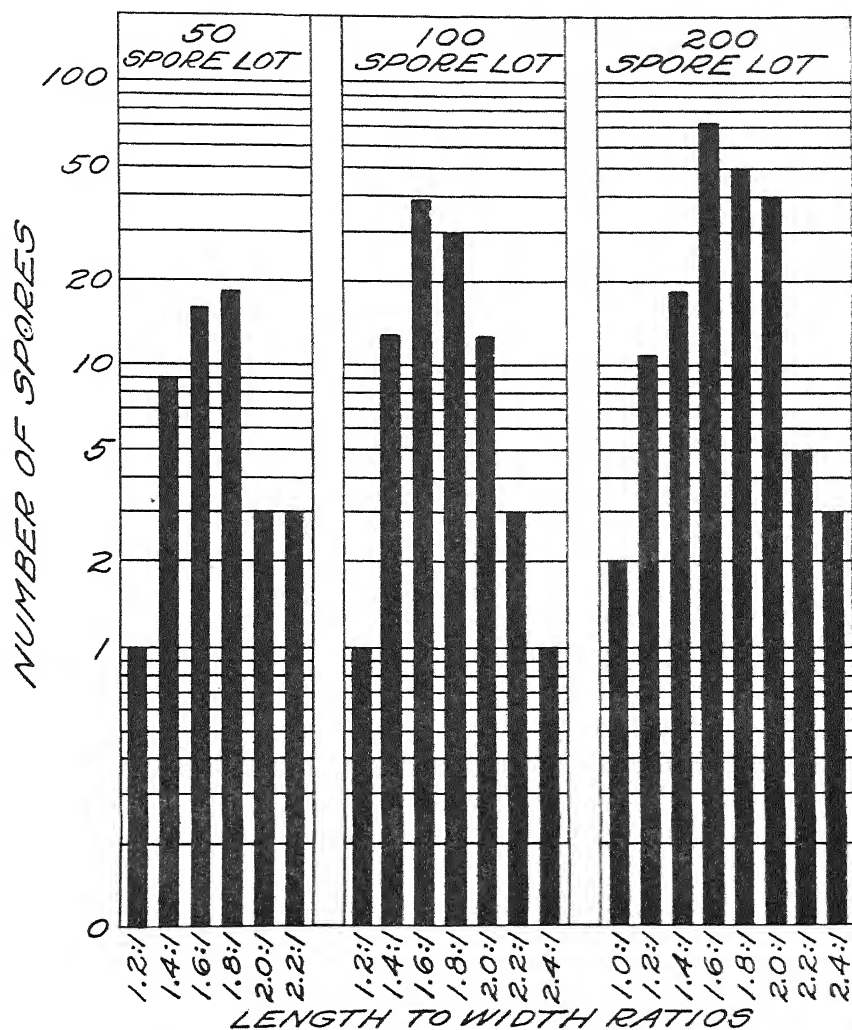


FIG. 6. Similarity in shape of urediniospores of independent random samples of different magnitude obtained from a single sorus of *Puccinia graminis tritici* form 1.

representative and adequate. To ensure that any error in the final analysis would be on the side of caution, the comparisons in the subsequent experiments were made on the basis of random samples consisting in each case of 100 spores, and only those differences are regarded as definitely significant which exceed their probable errors close to five times, *i.e.*, whose chance occurrence would be not more than one in a thousand trials.

Effect of Environmental Conditions.—How great an effect environmental and cultural conditions exert on the morphology of the physiologic forms of *P. graminis tritici* is another question which suggested itself in connection with the study of the comparative morphology of these forms. Monosporous cultures of forms 1 and 3 and a multisporous culture of form 27 were studied with this aim in view. The resulting data are presented in tables 18 to 21 and figures 10 to 18, both inclusive. It was found that the urediniospores of form 1 cultures confined in glass cages when the relative humidity was very high, were considerably longer than the urediniospores of the cultures kept exposed for a considerable length of time on the greenhouse bench. In both cases the infected hosts were the same and there

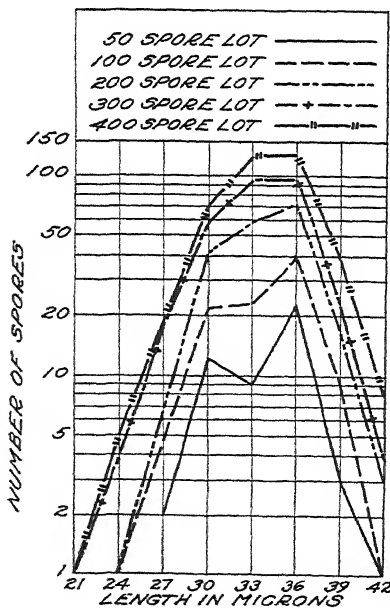


FIG. 7

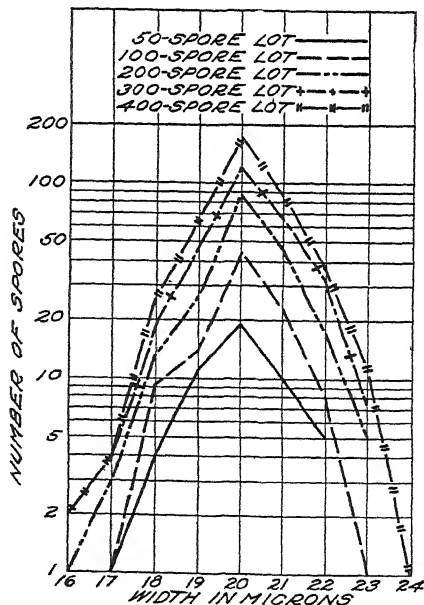


FIG. 8

FIG. 7. Similarity in length of urediniospores of composite random samples of different magnitude obtained from a single sorus of *Puccinia graminis tritici* form 1.

FIG. 8. Similarity in width of urediniospores of composite random samples of different magnitude obtained from a single sorus of *Puccinia graminis tritici* form 1.

was no detectible difference either in type or degree of infection. Only a slight decrease in spore size occurred when a confined culture was exposed for a short period of time (three days). This probably was not statistically significant, as the difference in the mean length was just three times greater than the probable error of the difference and the difference of the mean width was even smaller than its probable error. An etiolated condition of the host caused by an accidental contamination with mold affected the degree of infection somewhat and resulted in a profound decrease in the length and hence also in a change in the shape of the spores.

Urediniospores of form 3, developed on a congenial host (Little Club) and measured 15 days after inoculation in October, showed little difference either in size or shape, when compared with spores of the identical culture and of the same age of development measured in September. The prevailing climatic conditions in both months, as may be seen from table 22, were very much alike, which evidently accounts for the results obtained.

Even a semi-resistant host, such as Federation, appears to reduce the size of the urediniospores cultured on it, decreasing their mean length by

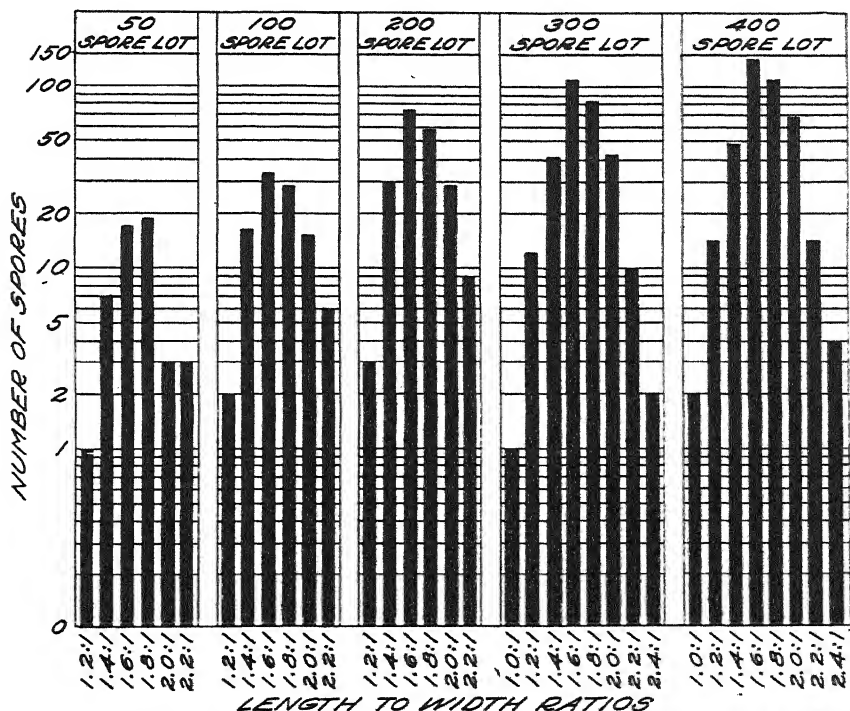


FIG. 9. Similarity in shape of urediniospores of composite random samples of different magnitude obtained from a single sorus of *Puccinia graminis tritici* form 1.

TABLE 18.—Variations and constants for length of aecidiospores of physiologic forms of *Puccinia graminis tritici* grown on different host plants, under diverse environmental conditions, and for varying lengths of time

Cultural conditions	Spore classes according to length							Size limits	Mean length	Constants		
	24μ 24μ 27μ 30μ 33μ 36μ 39μ 42μ									Standard deviation	Coefficient of variability	
	<i>Puccinia graminis tritici</i> form 1											
Rust inclosed, host vigorous...	1	1	12	33	34	15	1	25.07 — 43.24	31.77 ± 0.22	3.29 ± 0.16	9.46 ± 0.15	
Rust inclosed, host etiolated...	12	25	33	16	12	2		23.00 — 37.95	29.91 ± 0.25	3.73 ± 0.18	12.47 ± 0.59	
Confined 17 days, exposed 3 days		2	20	36	33	7	2	25.53 — 41.17	33.87 ± 0.20	2.98 ± 0.11	8.80 ± 0.42	
Confined 8 days, exposed 24 days	1	4	12	30	34	15	3	20.17 — 43.17	31.62 ± 0.21	3.61 ± 0.17	11.12 ± 0.54	
<i>Puccinia graminis tritici</i> form 3												
Cultured on Little Club during September	4	8	26	29	25	8		23.46 — 40.02	32.61 ± 0.23	3.38 ± 0.16	10.36 ± 0.49	
Cultured on Little Club during October	1	4	4	19	29	26	13	4	20.47 — 43.01	33.63 ± 0.28	4.21 ± 0.20	12.52 ± 0.60
Cultured on Federation during October	1	4	15	24	35	19	2	21.16 — 38.64	31.59 ± 0.24	3.56 ± 0.17	11.27 ± 0.54	
<i>Puccinia graminis tritici</i> form 27												
Spores measured 15 days after inoculation	1	18	32	33	13	2	1	19.78 — 38.18	28.47 ± 0.22	3.24 ± 0.15	11.38 ± 0.54	
Spores measured 20 days after inoculation	3	13	21	24	25	13	1	21.39 — 39.10	29.91 ± 0.28	4.09 ± 0.20	13.66 ± 0.65	

TABLE 19.—Variations and constants for width of urediniospores of *Puccinia graminis tritici* grown on different host plants, under diverse environmental conditions, and for varying lengths of time

Cultural conditions	Spore classes according to width										Size limits	Constants		
	15μ	16μ	17μ	18μ	19μ	20μ	21μ	22μ	23μ	24μ		Mean width	Standard deviation	Coefficient of variability
<i>Puccinia graminis tritici</i> form 1														
Rust inclosed, host vigorous				6	19	46	18	6	4	1	18.17 — 24.61	20.15 ± 0.08	1.16 ± 0.06	5.76 ± 0.27
Rust inclosed, host etiolated														
Confined 17 days, exposed 3 days		1	8	9	18	35	15	7	5	2	16.79 — 24.38	19.88 ± 0.11	1.62 ± 0.08	8.15 ± 0.39
Confined 8 days, exposed 24 days		1	2	4	12	45	23	9	4		16.33 — 23.23	20.23 ± 0.08	1.23 ± 0.06	6.08 ± 0.30
			1	3	18	31	27	15	5		17.25 — 23.46	20.45 ± 0.08	1.23 ± 0.06	6.01 ± 0.29
<i>Puccinia graminis tritici</i> form 3														
Cultured on Little Club during September ...		1	1	8	17	45	17	9	2		16.79 — 23.69	20.01 ± 0.08	1.20 ± 0.06	6.00 ± 0.29
Cultured on Little Club during October			3	17	24	35	14	6	1		17.25 — 23.23	19.62 ± 0.08	1.23 ± 0.06	6.27 ± 0.30
Cultured on Federation during October			7	15	32	33	9	3	1		17.02 — 23.00	19.35 ± 0.08	1.20 ± 0.06	6.20 ± 0.30
<i>Puccinia graminis tritici</i> form 27														
Spores measured 15 days after inoculation	3	3	6	18	30	21	12	7			15.18 — 22.77	19.15 ± 0.11	1.57 ± 0.07	8.20 ± 0.39
Spores measured 20 days after inoculation			4	15	26	38	14	2	1		17.02 — 23.00	19.53 ± 0.08	1.14 ± 0.05	5.84 ± 0.28

TABLE 20.—Variations and constants for shape of urediniospores of physiologic forms of *Puccinia graminis tritici* grown on different host plants, under diverse environmental conditions, and for varying lengths of time

Cultural conditions	Spore classes according to ratio of length to width								Correlation coefficient	Constants		Coefficient of variability	
										Mean ratio	Standard deviation		
	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4					
<i>Puccinia graminis tritici</i> form 1													
Rust inclosed, host vigorous	1	2	7	32	26	4	2		0.557 ± 0.017	1.768 ± 0.017	0.246 ± 0.012	13.91 ± 0.66	
Rust inclosed, host etiolated	1	20	23	31	19	4	1	1	- 0.126 ± 0.066	1.536 ± 0.017	0.254 ± 0.012	16.51 ± 0.79	
Confined 17 days, exposed 3 days		1	13	39	30	13	3	1	- 0.340 ± 0.060	1.708 ± 0.011	0.212 ± 0.010	12.41 ± 0.59	
Confined 8 days, exposed 24 days	2	13	15	14	18	7	1		- 0.273 ± 0.062	1.576 ± 0.016	0.235 ± 0.011	14.91 ± 0.71	
<i>Puccinia graminis tritici</i> form 3													
Cultured on Little Club during September	1	7	12	42	20	14	4		- 0.329 ± 0.060	1.662 ± 0.017	0.246 ± 0.012	14.80 ± 0.71	
Cultured on Little Club during October	2	6	8	29	31	18	4	2	- 0.336 ± 0.060	1.722 ± 0.018	0.271 ± 0.013	15.71 ± 0.75	
Cultured on Federation during October	2	11	11	31	26	16	3		- 0.483 ± 0.052	1.656 ± 0.018	0.270 ± 0.013	16.30 ± 0.78	
<i>Puccinia graminis tritici</i> form 27													
Spores measured 15 days after inoculation	3	20	26	34	11	3	2	1	- 0.308 ± 0.061	1.501 ± 0.017	0.258 ± 0.012	17.15 ± 0.82	
Spores measured 20 days after inoculation	3	19	17	32	17	11	1		- 0.295 ± 0.062	1.556 ± 0.018	0.272 ± 0.013	17.49 ± 0.83	

TABLE 21.—Summary of differences between the means of the dimensions of urediniospores of *Puccinia graminis tritici* grown on different host plants, under diverse environmental conditions, and for varying lengths of time

Cultural conditions compared	LENGTH		WIDTH		SHAPE	
	Difference in means (in microns)	Difference divided by P. E.	Difference in means (in microns)	Difference divided by P. E.	Difference in means (in microns)	Difference divided by P. E.
<i>Puccinia graminis tritici</i> form 1						
Rust inclosed, host vigorous and rust inclosed, host etiolated	4.80 ± 0.33	14.73	0.27 ± 0.14	1.93	0.232 ± 0.024	0.67
Confined 17 days, exposed 0 days and confined 17 days, exposed 3 days	0.80 ± 0.30	3.00	0.08 ± 0.11	0.73	0.060 ± 0.022	2.73
Confined 17 days, exposed 0 days and confined 8 days, exposed 24 days	3.15 ± 0.33	9.55	0.30 ± 0.11	2.73	0.192 ± 0.023	8.35
Confined 17 days, exposed 3 days and confined 8 days, exposed 24 days	2.25 ± 0.31	7.26	0.22 ± 0.11	2.00	0.132 ± 0.021	6.20
<i>Puccinia graminis tritici</i> form 3						
Cultured on Little Club in September and cultured on Little Club in October	1.02 ± 0.36	2.83	0.39 ± 0.11	3.55	0.060 ± 0.025	2.40
Cultured on Little Club in September and cultured on Federation in October	1.02 ± 0.33	3.09	0.66 ± 0.11	6.00	0.006 ± 0.025	0.24
Cultured on Little Club in October and cultured on Federation in October	2.04 ± 0.39	5.23	0.27 ± 0.11	2.45	0.066 ± 0.025	2.64
<i>Puccinia graminis tritici</i> form 27						
Measured 15 days after inoculation and measured 20 days after inoculation	1.47 ± 0.36	4.08	0.38 ± 0.14	2.71	0.052 ± 0.025	2.08

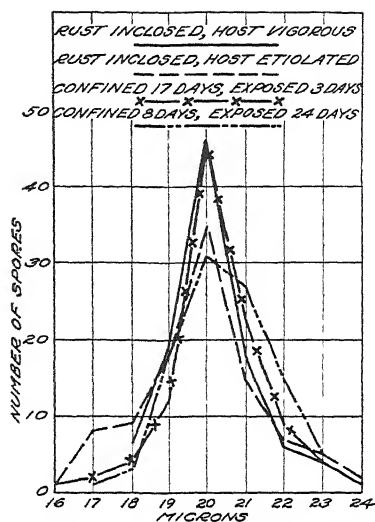
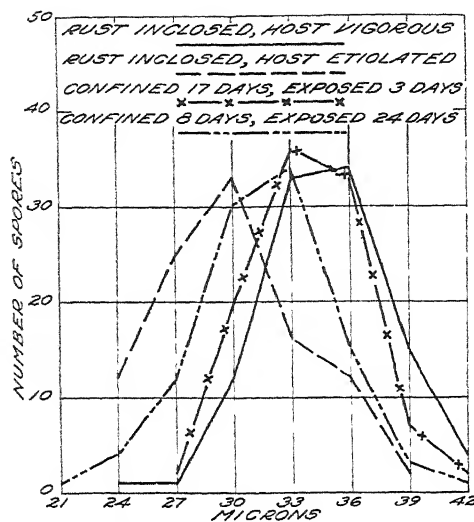


FIG. 10. Variation in length of urediniospores of *Puccinia graminis tritici* form 1, cultured under favorable and adverse environmental conditions.

FIG. 11. Variation in width of urediniospores of *Puccinia graminis tritici* form 1, cultured under favorable and adverse environmental conditions.

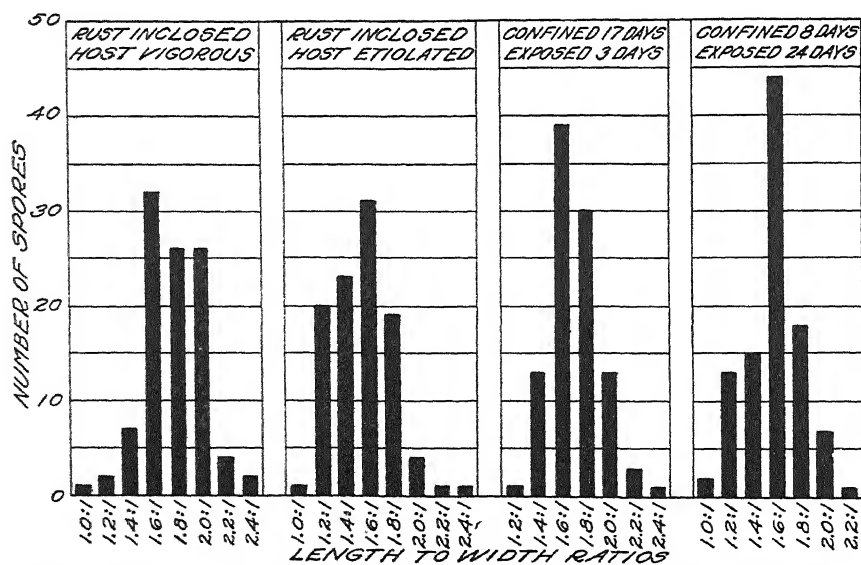


FIG. 12. Variation in shape of urediniospores of *Puccinia graminis tritici* form 1, cultured under favorable and adverse environmental conditions.

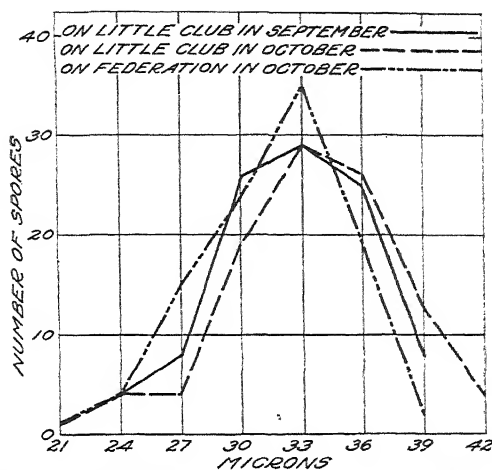


FIG. 13. Variation in length of urediniospores of *Puccinia graminis tritici* form 3, cultured at different times and on different hosts.

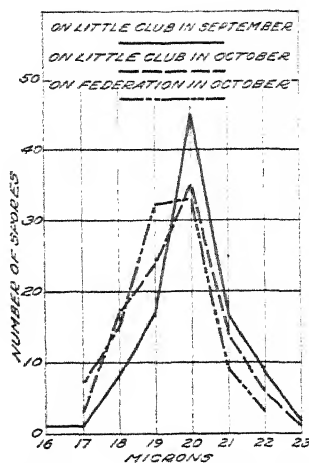


FIG. 14. Variation in width of urediniospores of *Puccinia graminis tritici* form 3, cultured at different times and on different hosts.

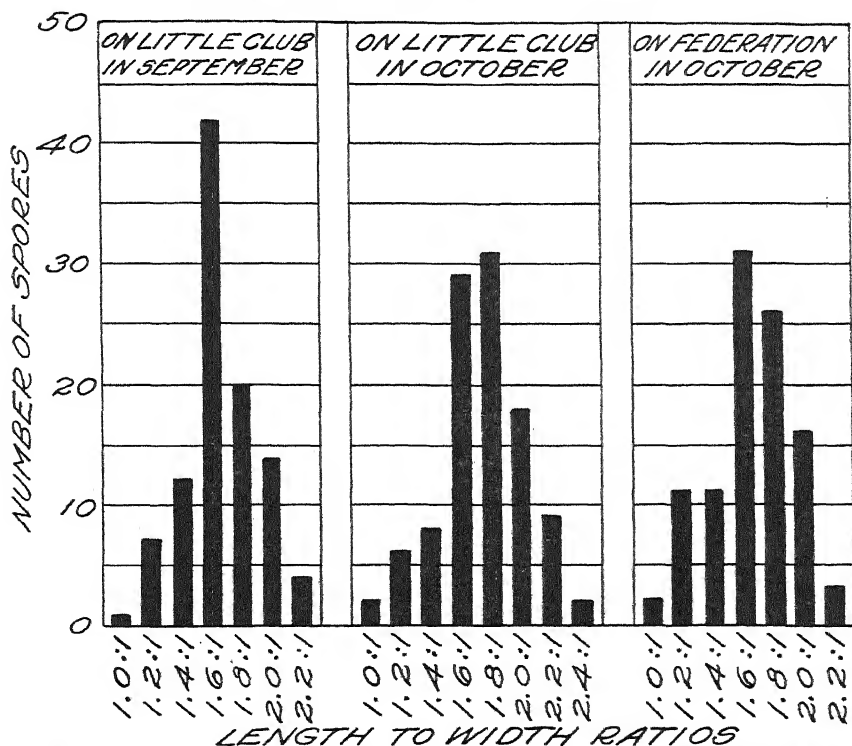


FIG. 15. Variation in shape of urediniospores of *Puccinia graminis tritici* form 3, cultured at different times and on different hosts.

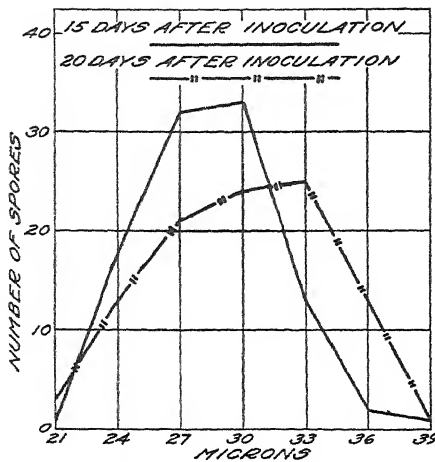


FIG. 16. Variation in length of urediniospores of *Puccinia graminis tritici* form 27, measured after different periods from date of inoculation.

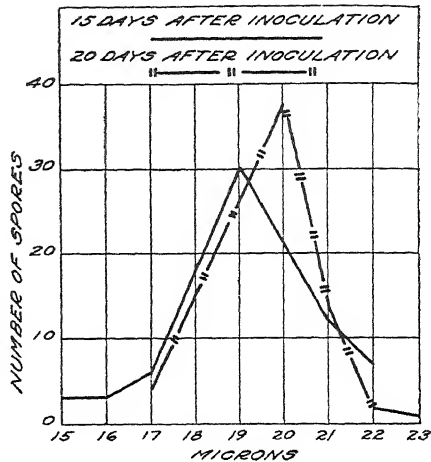


FIG. 17. Variation in width of urediniospores of *Puccinia graminis tritici* form 27, measured after different periods from date of inoculation.

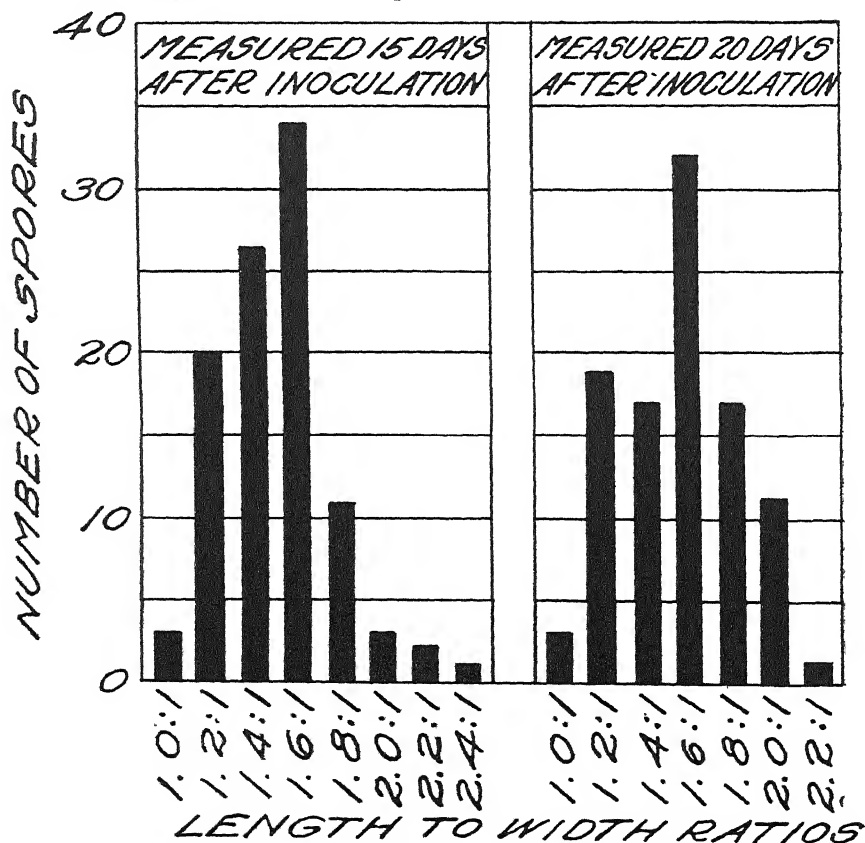


FIG. 18. Variation in shape of urediniospores of *Puccinia graminis tritici* form 27, measured after different periods from date of inoculation.

TABLE 22.—*Summary of prevailing temperature and light conditions at St. Paul, Minn., during September and October, 1924*

Weather conditions	September	October
<i>Temperature (Degrees Fahrenheit)</i>		
Maximum	65.2	65.7
Minimum	48.2	46.0
Mean	56.7	55.8
<i>Condition of Sky</i>		
No. days clear	10	14
No. days partly cloudy	10	10
No. days cloudy	10	7
<i>Sunshine</i>		
Per cent of possible	53	66

from 1 to 2 microns and their mean width by from $1/4$ to $2/3$ microns. In all cases these differences seem to be quite significant.

Maturity of the urediniospores also has considerable to do with their relative size. The data regarding the effect of ecological factors on the size and shape of urediniospores of physiologic forms of *P. graminis tritici* are presented in detail in tables 18 to 22 and in figures 10 to 18, inclusive.

Influence of Geographic Habitat.—Are physiologic forms of *P. graminis tritici*, identical in their parasitic behavior on the differential varieties of wheat, but of different geographical origin, similar or dissimilar in their morphologic structure? To gain knowledge concerning this point, a strain of form 3, collected in Utah in 1923, was compared with the prototype culture of this form, collected in Oklahoma in 1917. Likewise two strains of form 27, one from India, collected in 1923, and another from California, collected in 1924, were compared. The facts regarding these cultures are given in tables 23 to 26 and figures 19 to 24, inclusive.

There was a difference of 1.29 ± 0.39 microns in the mean length and 0.68 ± 0.11 in the mean width of the two strains of form 3 in favor of the Utah culture. Both of these differences very probably are significant. However, the two strains were studied at different times, and other factors may have played a part, which might account for the differences. On the other hand, the differences may be genetic in nature and due perhaps to the fact that on other differential hosts the two strains might be shown to be distinct physiologic forms. There also is the possibility that even though the two strains possessed the same parasitic capabilities their morphologic evolution in Utah and California might have been different.

In the case of form 27 the two strains were grown on identical hosts (Vernal emmer), inoculated on the same date and measured on the same day. And yet, although there was practically no difference in the mean

TABLE 23.—Variations and constants for length of urediniospores of phyllostic forms of *Puccinia graminis tritici*, originated under separate geographic conditions

Place of origin	Spore classes according to length						Size limits	Mean	Constants Standard deviation	Coefficient of variability
	21μ	24μ	27μ	30μ	33μ	36μ				
<i>P. graminis tritici</i> form 3										
Sulwater, Okla.	3	12	25	31	22	7	23.68	40.00	3.61 ± 0.17	11.16 ± 0.53
Lehi, Utah	1	4	19	29	26	13	4	20.47 — 43.01	33.63 ± 0.28	12.52 ± 0.60
<i>P. graminis tritici</i> form 27										
Pusa, India	2	10	21	27	25	11	20.47	37.72	3.79 ± 0.18	12.63 ± 0.60
Berkeley, Calif.	3	13	21	24	25	13	4	21.39 — 39.10	29.94 ± 0.28	13.66 ± 0.65

TABLE 24.—Variations and constants for width of urediniospores of phyllostic forms of *Puccinia graminis tritici*, originated under separate geographic conditions

Place of origin	Spore classes according to width						Size limits		Mean	Constants	Coefficient of variability			
	16μ	17μ	18μ	19μ	20μ	21μ	22μ	23μ	24μ	Standard deviation				
	<i>P. graminis tritici</i> form 3													
Stillwater, Okla.	1	6	21	48	18	6		16.96	21.76	18.94 ± 0.07	0.98 ± 0.05	5.17 ± 0.25		
Lehi, Utah		3	17	24	35	14	6	1	17.25	23.23	19.62 ± 0.08	1.23 ± 0.06	6.27 ± 0.30	
	<i>P. graminis tritici</i> form 27													
Pusa, India		3	10	27	18	18	11	11	2	17.25	24.84	20.25 ± 0.11	1.68 ± 0.08	8.30 ± 0.10
Berkeley, Calif.		4	15	26	38	14	2	1		17.02	23.00	19.53 ± 0.08	1.74 ± 0.05	5.84 ± 0.28

TABLE 25.—Variations and constants for shape of urediniospores of physiologic forms of *Puccinia graminis tritici*, originated under separate geographic conditions

Place of origin	Spore classes according to ratio of length to width								Correlation coefficient	Constants		Coefficient of variability
										Mean ratio	Standard deviation	
	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4				
<i>Puccinia graminis tritici</i> form 3												
Stillwater, Okla.		6	9	29	30	18	8		-0.435 ± 0.055	1.738 ± 0.017	0.253 ± 0.012	14.56 ± 0.69
Lehi, Utah	2	6	8	29	31	18	4	2	-0.336 ± 0.060	1.722 ± 0.018	0.271 ± 0.013	15.74 ± 0.75
<i>Puccinia graminis tritici</i> form 27												
Pusa, India	1	18	28	34	15	3	1		+0.212 ± 0.064	1.514 ± 0.015	0.225 ± 0.011	15.91 ± 0.76
Berkeley, Calif.	3	19	17	32	17	11	1		-0.295 ± 0.062	1.556 ± 0.018	0.272 ± 0.013	17.49 ± 0.83

TABLE 26.—Summary of differences between the means of the dimensions of urediniospores of physiologic forms of *Puccinia graminis tritici*, originated under separate geographic conditions

Physiologic forms of diverse origin compared	LENGTH			WIDTH			SHAPE	
	Difference in means (in microns)	Difference divided by P. E.		Difference in means (in microns)	Difference divided by P. E.		Difference in means (in microns)	Difference divided by P. E.
<i>Puccinia graminis tritici</i> form 3								
Form 3, Oklahoma strain, and form 3, Utah strain	1.29 ± 0.39	3.31		0.68 ± 0.11	6.18		0.016 ± 0.025	0.64
<i>Puccinia graminis tritici</i> form 27								
Form 27, India strain, and form 27, California strain	0.06 ± 0.38	0.16		0.72 ± 0.14	5.14		0.012 ± 0.023	1.83

length of spores of the two strains ($0.06 \pm 0.38 \mu$), the mean width of the spores of the India strain was $0.72 \pm 0.14 \mu$ greater than that of the California strain, a difference which exceeds its probable error 5.14 times and is not likely to be due to random sampling. Here again arises the question of the effect of origin on the morphology of presumably identical strains of a given physiologic form. It would not be safe to draw any definite conclusions from this experiment, for even here some environmental factor, possibly light, may have affected the result. The India strain was grown on the bench, near the outer glass wall of the greenhouse, while the California strain was on the opposite side, facing a brick wall. The situation needs to be reversed and further studies made in order to gain conclusive knowledge.

It is interesting to note in this connection that there was no appreciable difference in the shape of the two strains of form 3; likewise there appeared to be no significant biometrical difference in the shape of spores of the two strains of form 27.

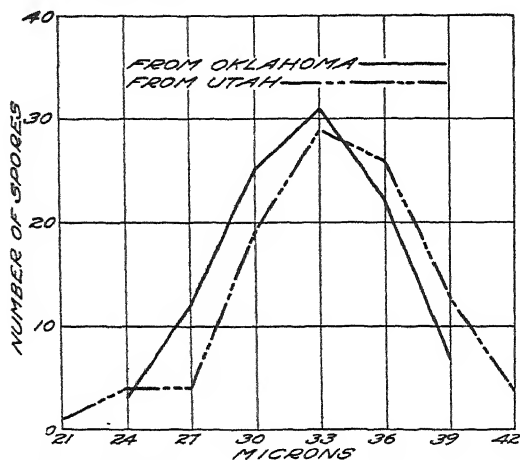


FIG. 19. Variation in length of urediniospores of *Puccinia graminis tritici* form 3, originated under diverse geographic conditions.

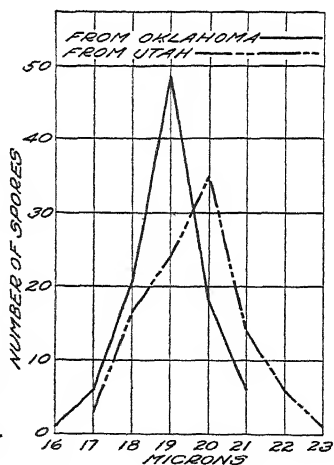


FIG. 20. Variation in width of urediniospores of *Puccinia graminis tritici* form 3, originated under diverse geographic conditions.

Comparative Anatomy Under Similar Conditions.—Four of the eight forms selected for the comparative morphological study (forms 1, 3, 9, and 17) were of monosporous origin and were cultured in closed, spore-proof, glass cages. The other four (forms 15, 27, 29, and 38) were multi-sporous stock cultures, grown in open compartments on the greenhouse benches. Otherwise the forms were cultured under virtually identical conditions and on congenial hosts. Form 15 was chosen because of its extreme

virulence, being able to infect heavily all the differential hosts of *Puccinia graminis tritici* except Khapli. Form 27, on the other hand, was chosen because it is the weakest physiologic form of the wheat stem rust now available; it produces normal infection only on Little Club, Kubanka, Acme, and Vernal emmer. Form 1 is the oldest culture on hand and attacks the hard red spring wheats very heavily, but half of the differential hosts are highly resistant to it. Forms 3, 9, 17, and 29 occur very frequently, are widely distributed, and rather virulent. Form 38, like form 29, produces a heterogeneous type of infection on the durum varieties. Marquis is very resistant to form 38 and Kanred is completely susceptible to it; the reverse is true for form 29.

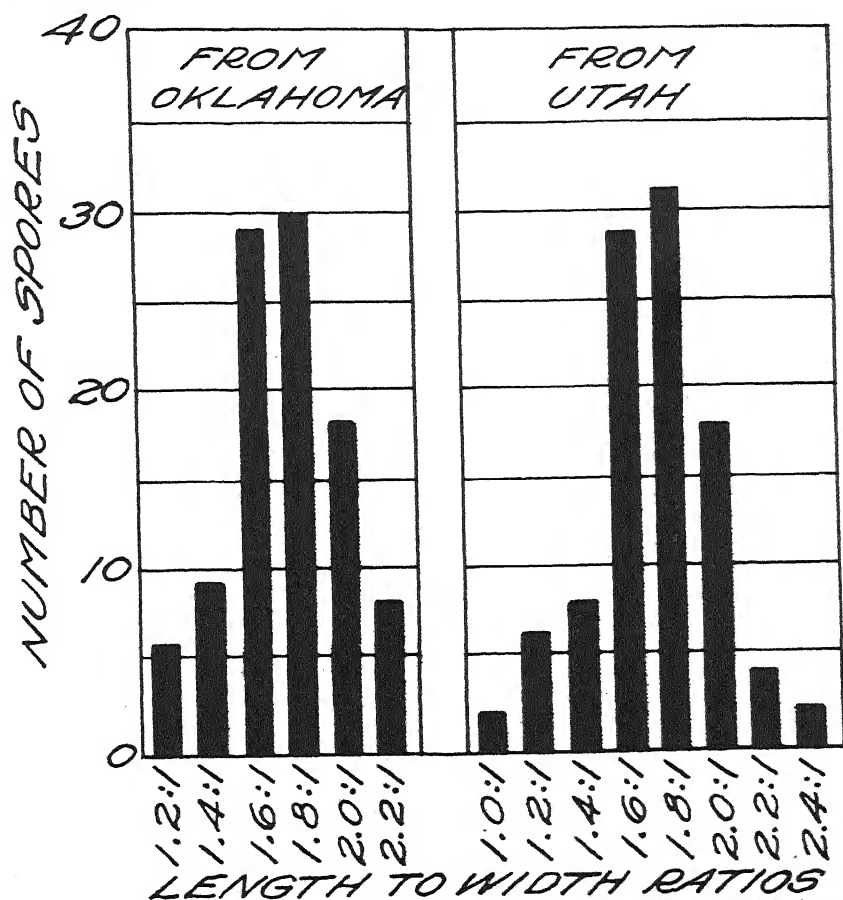


FIG. 21. Variation in shape of urediniospores of *Puccinia graminis tritici* form 3, originated under diverse geographic conditions.

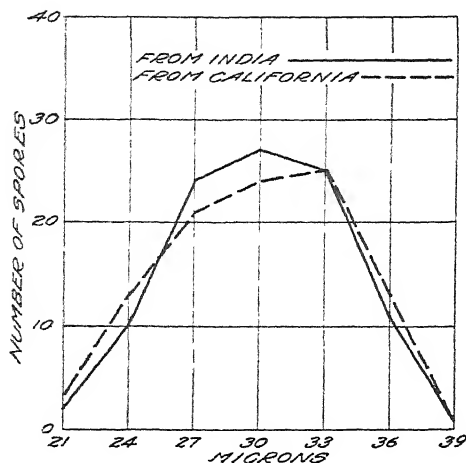


FIG. 22. Variation in length of urediniospores of *Puccinia graminis tritici* form 27, originated under diverse geographic conditions.

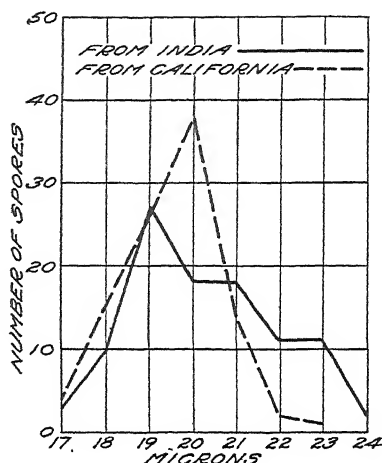


FIG. 23. Variation in width of urediniospores of *Puccinia graminis tritici* form 27, originated under diverse geographic conditions.

These forms were found to differ significantly, in part or in whole, not only in their pathogenicity, but also in the size and shape of their urediniospores, and, to a certain extent, also in the correlation between the length and width of these spores and their morphogenic processes when studied under uniform environmental conditions (See tables 27-30 and figures 25 to 30, inclusive).

For the sake of convenience, the urediniospores in the following discussion are considered as belonging to five classes, according to shape, viz.:

1. Spherical, embracing spores with ratios approximating..... 1.0 : 1
2. Spheroidal, comprising individuals within ratio classes.... 1.0 : 1 to 1.2 : 1
3. Subglobose, do 1.4 : 1 to 1.6 : 1
4. Ellipsoid, do 1.8 : 1 to 2.0 : 1
5. Elliptical, do 2.2 : 1 to 2.4 : 1

P. graminis tritici form 1.—The urediniospores of form 1 (Table 31), on the average, are significantly longer than those of forms 9 and 17. They also are considerably broader than the urediniospores of either form 3 or form 9 but are quite similar in width to those of form 17:1. Their mean measurements are $34.77 \pm 0.22 \times 20.15 \pm 0.08 \mu$. In shape, the urediniospores of form 1 are predominantly elongate-ovate, or elliptical, and ellipsoid, their mean length-to-width ratio being 1.768 ± 0.017 . In shape as well as in size the urediniospores of form 1 are the least variable among the monosporous forms. There appears to be a definite negative correlation of

0.557 ± 0.047 between the long and short diameters of these spores (the highest correlation among all the forms studied), but the percentage of variability does not exceed 17 per cent (16.95 per cent to be exact). This, however, is the highest percentage of variability encountered in this study. The percentage of variability controlled by the correlation, r , was determined by the formula $100 \times (1 - \sqrt{1 - r^2})$.

P. graminis tritici form 3.—The mean dimensions of the urediniospores of form 3 (Table 32) are $33.63 \pm 0.28 \times 19.62 \pm 0.08 \mu$; on the average, $1.14 \pm 0.36 \mu$ shorter and $0.53 \pm 0.11 \mu$ narrower than those of form 1.

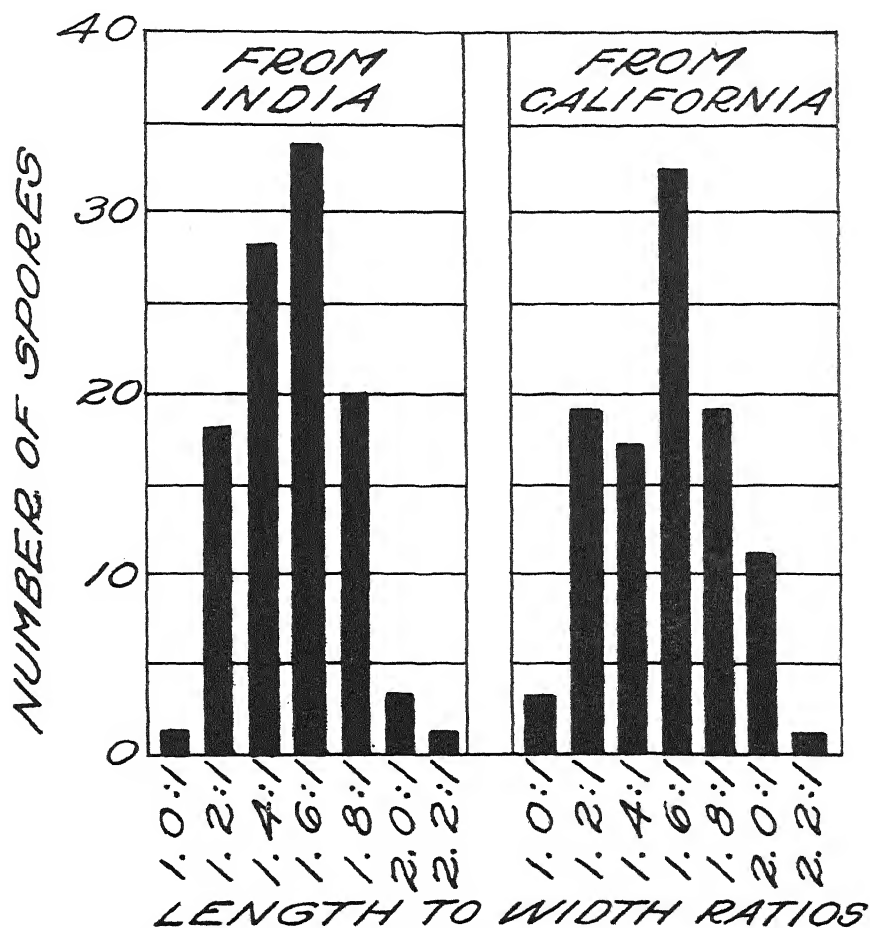


FIG. 24. Variation in shape of urediniospores of *Puccinia graminis tritici* form 27, originated under diverse geographic conditions.

TABLE 27.—Variations and constants for length of uredinospores of *Puccinia graminis tritici*, grown on congenial host plants and under uniform environmental conditions

Type of culture	Form	Spore classes according to length							Size limits	Mean length	Constants	
		24 μ	25 μ	27 μ	30 μ	33 μ	36 μ	39 μ	42 μ		Standard deviation	Coefficient of variability
MONOSPOROTS	1	1	1	12	33	34	15	4	25.07 — 43.24	34.77 \pm 0.22	3.29 \pm 0.16	9.46 \pm 0.45
	3	1	4	4	19	29	26	13	20.47 — 43.01	33.63 \pm 0.28	4.21 \pm 0.20	12.52 \pm 0.60
	9	1	3	14	35	28	17	2	21.62 — 40.35	31.35 \pm 0.23	3.40 \pm 0.16	10.85 \pm 0.52
	17	1	7	26	40	24	2		22.08 — 36.57	29.55 \pm 0.19	2.89 \pm 0.14	9.78 \pm 0.47
MULTISPOROTS	15	1	2	11	22	36	26	2	22.08 — 39.10	32.28 \pm 0.23	3.40 \pm 0.16	10.53 \pm 0.50
	27	3	13	21	24	25	13	1	21.39 — 39.10	29.91 \pm 0.28	4.09 \pm 0.20	13.66 \pm 0.65
	29	2	11	31	41	14	1		20.47 — 35.65	28.71 \pm 0.19	2.89 \pm 0.14	10.07 \pm 0.48
	38		2	17	30	34	14	3	24.15 — 39.79	31.50 \pm 0.22	3.24 \pm 0.15	10.29 \pm 0.49

TABLE 28.—Variations and constants for width of urediniospores of *Puccinia graminis tritici* grown on congenial host plants under uniform environmental conditions

Type of culture	Form	Spore classes according to width										Size limits	Constants		
													Mean width	Standard deviation	Coefficient of variability
		16 μ	17 μ	18 μ	19 μ	20 μ	21 μ	22 μ	23 μ	24 μ	25 μ				
MONOSPORES	1			6	19	46	18	6	4	1		18.17 — 24.61	20.15 \pm 0.08	1.16 \pm 0.06	5.76 \pm 0.27
	3		3	17	24	35	14	6	1			17.25 — 23.23	19.62 \pm 0.08	1.23 \pm 0.06	6.27 \pm 0.30
	9	2	7	23	30	23	9	5	1			16.56 — 23.46	19.17 \pm 0.09	1.36 \pm 0.06	7.09 \pm 0.34
	17	1	1	4	16	36	30	10	1	1		16.10 — 24.38	20.26 \pm 0.08	1.20 \pm 0.06	5.92 \pm 0.28
MULTISPORES	15				2	13	24	32	20	7	2	19.32 — 25.99	21.84 \pm 0.08	1.25 \pm 0.06	5.72 \pm 0.27
	27		4	15	26	38	14	2	1			17.02 — 23.00	19.53 \pm 0.08	1.14 \pm 0.05	5.84 \pm 0.28
	29		5	11	38	35	8	2	1			17.71 — 23.69	19.40 \pm 0.07	1.08 \pm 0.05	5.57 \pm 0.27
	38		1	7	20	32	30	9	1			17.94 — 23.23	20.14 \pm 0.08	1.14 \pm 0.05	5.66 \pm 0.27

TABLE 20.—*Variations and constants for shape of conidiospores of Puccinia graminis tritici, grown on congenial host plants and under uniform environmental conditions*

Type of culture	Form	Spore classes according to ratio of length to width										Correlation coefficient	Constants		
		1.0	1.2	1.1	1.6	1.8	2.0	2.2	2.4				Mean ratio	Standard deviation	Coefficient of variability
MONOSPOROUS	1	1	2	7	32	26	26	4	2			-0.557 ± 0.047	1.768 ± 0.017	0.246 ± 0.012	13.91 ± 0.66
	3	2	6	8	29	31	18	4	2			-0.336 ± 0.060	1.722 ± 0.018	0.271 ± 0.013	15.74 ± 0.75
	9	3	8	11	37	24	11	6				-0.522 ± 0.049	1.656 ± 0.017	0.252 ± 0.012	15.21 ± 0.73
	17	2	24	27	33	13	1					-0.397 ± 0.057	1.468 ± 0.014	0.214 ± 0.010	15.27 ± 0.73
MULTISPOROUS	15	4	12	25	42	14	3					-0.357 ± 0.059	1.518 ± 0.015	0.217 ± 0.010	14.29 ± 0.69
	27	3	19	17	32	17	11	1				-0.295 ± 0.062	1.556 ± 0.018	0.272 ± 0.013	17.49 ± 0.83
	29	2	15	29	41	11	2					-0.259 ± 0.063	1.500 ± 0.014	0.201 ± 0.010	13.40 ± 0.64
	38	10	24	36	22	6	2					-0.003 ± 0.067	1.592 ± 0.015	0.225 ± 0.011	14.13 ± 0.67

TABLE 30.—Summary of differences between the means of the dimensions of urediniospores of *Puccinia graminis* tritici, grown on congenial host plants and under uniform environmental conditions

Type of culture	Physiologic forms compared	LENGTH		WIDTH		SHAPE	
		Difference in means (in microns)	Difference divided by P. E.	Difference in means (in microns)	Difference divided by P. E.	Difference in means (in microns)	Difference divided by P. E.
MONOSPORES	1 and 3	1.14 ± 0.36	3.17	0.53 ± 0.11	4.82	0.046 ± 0.025	1.84
	1 and 9	3.42 ± 0.32	10.69	0.98 ± 0.12	8.17	0.112 ± 0.024	4.67
	1 and 17	5.22 ± 0.29	17.99	0.11 ± 0.11	1.00	0.300 ± 0.022	13.64
	3 and 9	2.28 ± 0.36	6.33	0.45 ± 0.12	3.75	0.066 ± 0.025	2.64
	3 and 17	4.08 ± 0.34	12.00	0.61 ± 0.11	5.82	0.254 ± 0.023	11.04
	9 and 17	1.80 ± 0.30	6.00	1.09 ± 0.12	9.08	0.188 ± 0.022	8.55
MULTISPORES	15 and 27	2.34 ± 0.36	6.50	2.31 ± 0.11	21.00	0.038 ± 0.023	1.65
	15 and 29	3.57 ± 0.30	11.90	2.44 ± 0.31	22.18	0.018 ± 0.021	0.86
	15 and 38	0.78 ± 0.32	2.44	1.70 ± 0.11	15.45	0.074 ± 0.021	3.52
	27 and 29	1.23 ± 0.34	3.62	0.13 ± 0.11	1.18	0.056 ± 0.023	2.43
	27 and 38	1.56 ± 0.36	4.33	0.61 ± 0.11	5.55	0.036 ± 0.023	1.57
	29 and 38	2.70 ± 0.29	9.62	0.74 ± 0.11	6.73	0.092 ± 0.021	4.38

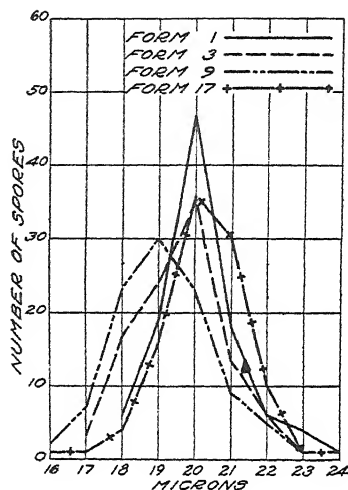
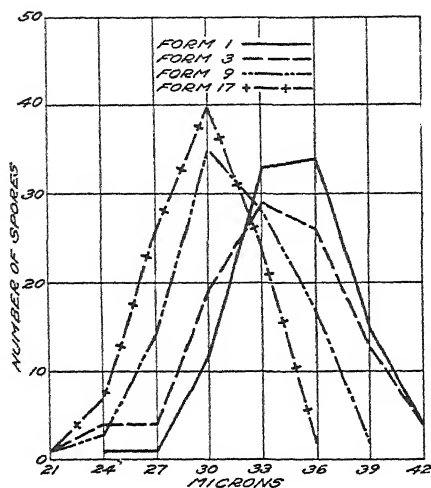


FIG. 25. Differences in urediniospore length of monosporous cultures of four physiologic forms of *Puccinia graminis tritici*, grown on congenial host plants and under uniform environmental conditions.

FIG. 26. Differences in urediniospore width of monosporous cultures of four physiologic forms of *Puccinia graminis tritici*, grown on congenial host plants and under uniform environmental conditions.

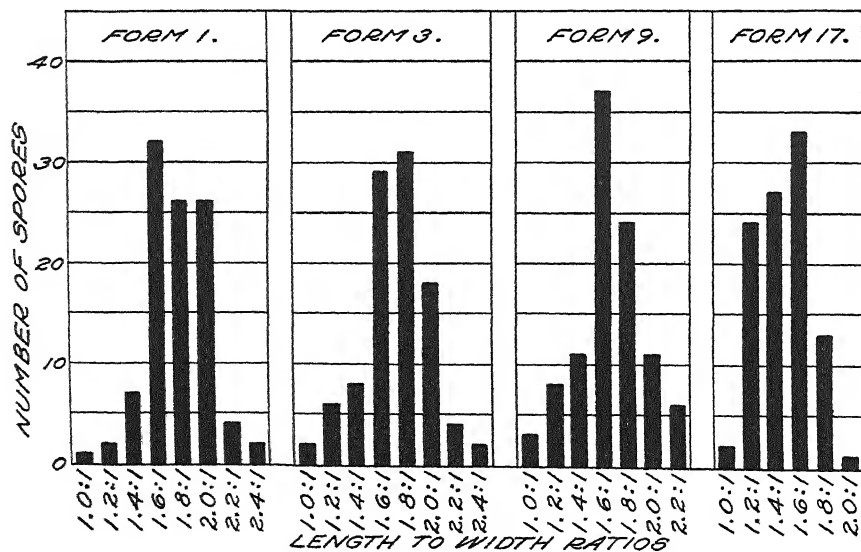


FIG. 27. Differences in urediniospores shape of monosporous cultures of four physiologic forms of *Puccinia graminis tritici*, grown on congenial host plants and under favorable environmental conditions.

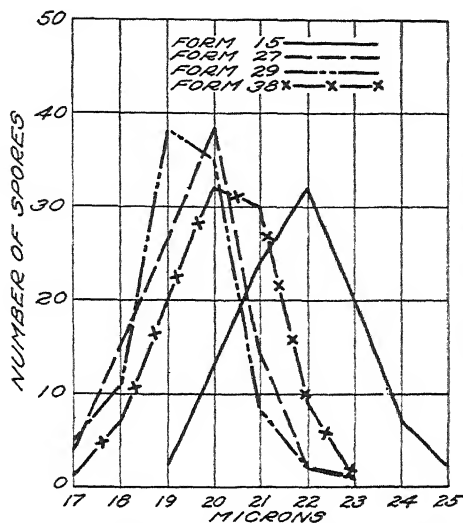


FIG. 28. Differences in urediniospore length of multispore cultures of four physiologic forms of *Puccinia graminis tritici*, grown on congenial host plants and under uniform environmental conditions.

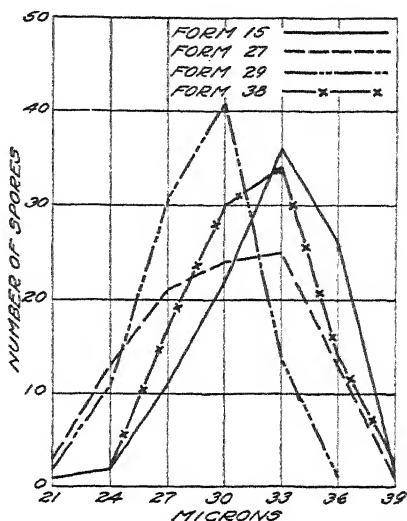


FIG. 29. Differences in urediniospore width of multispore cultures of four physiologic forms of *Puccinia graminis tritici*, grown on congenial host plants and under uniform environmental conditions.

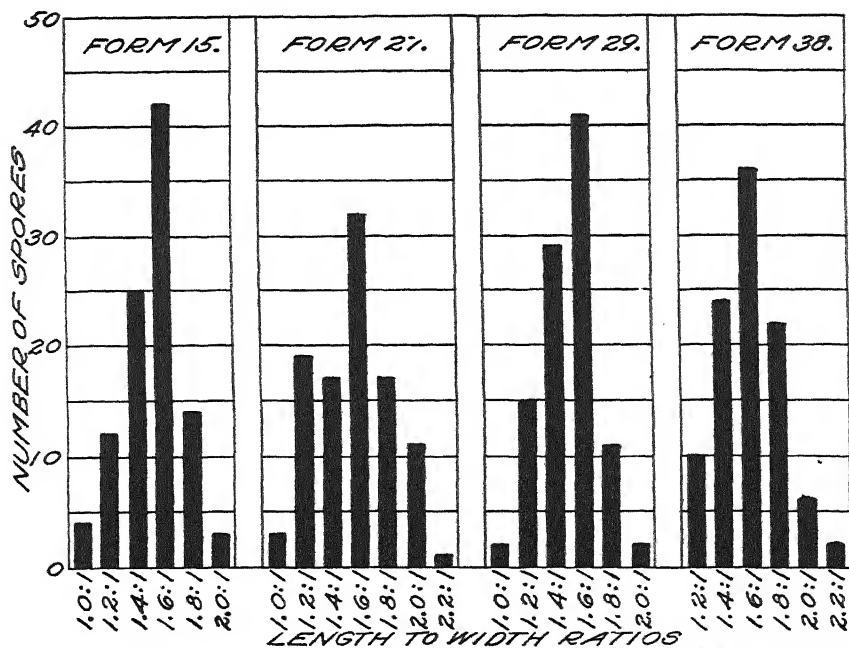


FIG. 30. Differences in urediniospore shape of multispore cultures of four physiologic forms of *Puccinia graminis tritici*, grown on congenial host plants

TABLE 31.—Correlation table for the length and width of 100 urediniospores of *Puccinia graminis tritici*, form 1. Spore width, subject; spore length, relative. Coefficient of correlation = -0.557 ± 0.047 ; control of variability = 16.95 per cent

		SPORE LENGTH							
		24μ	27μ	30μ	33μ	36μ	39μ		42μ
SPORE WIDTH	18μ					2	2	2	6
	19μ				5	11	2	1	19
	20μ			5	15	14	11	1	46
	21μ		1	2	8	7			18
	22μ			2	4				6
	23μ	1		2	1				4
	24μ			1					1
		1	1	12	33	34	15	4	100

TABLE 32.—Correlation table for the length and width of 100 urediniospores of *Puccinia graminis tritici* form 3. Spore width, subject; spore length, relative. Coefficient of correlation = -0.336 ± 0.060 ; control of variability = 5.81 per cent

		SPORE LENGTH								
		21μ	24μ	27μ	30μ	33μ	36μ	39μ	42μ	
SPORE WIDTH	17μ					1		2		3
	18μ				2	5	7	1	2	17
	19μ			1	3	8	8	3	1	24
	20μ	1	1		7	13	7	6		35
	21μ		2	2	5	2	2	1		14
	22μ		1	1	1		2		1	6
	23μ				1					1
		1	4	4	19	29	26	13	4	100

There is a greater variability in the length of urediniospores of form 3 than there is in those of either form 1, form 9, or form 17. The difference in length, although larger numerically than the difference in the width, perhaps is not significant statistically, as it exceeds its probable error only 3.17 times, the odds against the chance occurrence being about 30 to 1. But the difference between the mean widths of the two forms is 4.82 times greater than its probable error, and the odds are more than 800 to 1 that this difference is not due to random sampling. Therefore the difference in the spore width of the two forms very probably is significant. The shape of the urediniospores of form 3 is very much the same as that of the spores of form 1, their mean ratios being quite similar: $1.722 \pm 0.018:1$ as compared with $1.768 \pm 0.017:1$. Both forms have an equal number of elliptical urediniospores, but form 3 has a much greater proportion of spheroidal spores. Also there is a considerable greater variability in the shape of urediniospores of form 3 than there is in those of form 1. The correlation coefficient for the two diameters of form 3 is 0.336 ± 0.060 . The morphogenic process is affected by one of the diameters to the extent of only 5.81 per cent, almost two-thirds less than in the case of form 1.

P. graminis tritici form 9.—Form 9 (Table 33) has shorter as well as narrower spores than either form 1 or form 3, measuring $31.35 \pm 0.23 \times$

TABLE 33.—Correlation table for the length and width of 100 urediniospores of *Puccinia graminis tritici* form 9. Spore width, subject; spore length, relative. Coefficient of correlation = -0.522 ± 0.949 ; control of variability = 14.71 per cent

	SPORE LENGTH						
	21 μ	24 μ	27 μ	30 μ	33 μ	36 μ	39 μ
16 μ				2			2
17 μ				1		5	1
18 μ				8	10	5	23
19 μ	1		3	11	9	5	30
20 μ			4	9	8	2	23
21 μ			6	3			9
22 μ		2	1	1	1		5
23 μ		1					1
	1	3	14	35	28	17	2
							100

$19.17 \pm 0.09 \mu$. The differences in the mean length and mean width between the urediniospores of form 9 and form 1 are 10.69 and 8.17 times greater than their respective probable errors and undoubtedly are significant in both cases. The differences in these means between forms 9 and 3 exceed 6.33 and 3.75 times their respective probable errors, certainly a significant difference in length, but perhaps not in width. The urediniospores of form 9 are mostly ellipsoid and subglobose; the mean length-to-width ratio ($1.656 \pm 0.017:1$) being lower than that of either of the two forms mentioned above, but the variability in shape is greater than that of form 1 but somewhat smaller than that of form 3. The correlation between the long and short dimensions is negative, the correlation coefficient approaching that of form 1, namely, -0.522 ± 0.049 . The growth of one diameter as affected by the other amounts to 14.71 per cent.

P. graminis tritici form 17.—The shape of the urediniospores of form 17 (Table 34) is more characteristic than their size, there being a preponder-

TABLE 34.—Correlation table for length and width of 100 urediniospores of *Puccinia graminis tritici* form 17. Spore width, subject; spore length, relative. Coefficient of correlation = -0.397 ± 0.057 ; control of variability = 8.22 per cent

	SPORE LENGTH					
	21 μ	24 μ	27 μ	30 μ	33 μ	36 μ
SPORE WIDTH	16 μ				1	1
	17 μ			1		1
	18 μ				4	4
	19 μ	1	2	7	6	16
	20 μ	4	6	13	11	36
	21 μ	1	10	16	2	30
	22 μ	1	6	3		10
	23 μ		1			1
	24 μ		1			1
	1	7	26	40	24	2
						100

ance of subglobose and spheroidal individuals. Compared with the first three forms, just discussed, there is a marked and significant reduction in the mean length of the urediniospores of form 17. This reduction ranges from $1.80 \pm 0.30 \mu$ to $5.22 \pm 0.29 \mu$. The mean width, though, is almost identical with that of spores of form 1 but significantly different from the mean width of spores of forms 3 and 9. The dimensions of the urediniospores of form 17 are $29.55 \pm 0.19 \times 20.26 \pm 0.08 \mu$, and the mean length-to-width ratio is $1.468 \pm 0.014:1$. There are more spheroidal urediniospores in this form than in the other three monosporous forms combined. The coefficient of correlation (-0.397 ± 0.057) is more like that of form 3, but the per cent of variability, 8.22 per cent, is somewhat larger than in form 3 and much smaller than in forms 1 and 9.

P. graminis tritici form 15.—Inasmuch as form 15 (Table 35) and the subsequent forms were cultured under different conditions than the forms discussed above, it would not seem justifiable to compare the various members comprising these two groups. The present investigation has shown, however, that the urediniospores cultured outside of the spore cages generally were considerably smaller than those cultured within (See tables 18–21 and figures 10–12). With this in mind it will be interesting to note that the

TABLE 35.—Correlation table for length and width of 100 urediniospores of *Puccinia graminis tritici* form 15. Spore width, subject; spore length, relative. Coefficient of correlation = -0.357 ± 0.059 ; control of variability = 6.59 per cent

		SPORE LENGTH							
		21μ	24μ	27μ	30μ	33μ	36μ	39μ	
SPORE WIDTH	19μ						2	2	
	20μ			1	1	6	4	1	13
	21μ	1		1	5	7	9	1	24
	22μ		1	3	5	17	6		32
	23μ			3	8	4	5		20
	24μ		1	2	3	1			7
	25μ			1		1			2
		1	2	11	22	36	26	2	100

urediniospores of form 15, although developed in an open compartment, were much broader than those of any other form studied, whether cultured in this way or otherwise. The urediniospores of form 15 have a mean length and width of 32.28 ± 0.21 and $21.84 \pm 0.08 \mu$, respectively. The excess in the mean width of these spores over those of the other forms ranges from $1.58 \pm 0.11 \mu$ to $2.67 \pm 0.12 \mu$, these differences being from 14.36 to 22.25 times greater than their respective probable errors and obviously really significant. The mean ratio of length-to-width of the urediniospores of form 15 is $1.518 \pm 0.015:1$, only slightly larger than the ratio for form 17, notwithstanding the marked difference in the spore size of these two forms. However, form 15 possesses fewer spheroidal and more subglobose spores than does form 17. Both forms have approximately the same number of ellipsoid urediniospores and neither one has any elliptical spores. The correlation coefficient of form 15 (-0.357 ± 0.059) is not greatly different from that of form 17; nor is the effect on the morphogenic process of the two forms at great variance, 6.59 per cent for form 15 and 8.22 per cent for form 17.

P. graminis tritici form 27.—The urediniospores of form 27 (Table 36) averaging 29.04 ± 0.28 $19.53 \pm 0.08 \mu$, are both shorter and narrower than

TABLE 36.—Correlation table for length and width of 1% urediniospores of *Puccinia graminis tritici* form 27. Spore width, subject; spore length, relative. Coefficient of correlation = -0.245 ± 0.062 ; control of variability = $\frac{1}{4}$ per cent

	SPORE LENGTH						
	21μ	24μ	27μ	30μ	33μ	36μ	39μ
17μ	1	1	1		1		4
18μ		1	1	2	5	5	1
19μ		2	4	6	9	5	
20μ	2	4	7	12	10	3	
21μ		4	7	3			
22μ			1	1			
23μ		1					
	3	13	21	24	25	13	1

those of form 15. There is a difference of $2.34 \pm 0.36 \mu$ in length and $2.31 \pm 0.11 \mu$ in width. These differences undoubtedly are highly significant, as they are 6.50 and 21.00 times greater than their respective probable errors. The mean length-to-width ratio of form 27 ($1.556 \pm 0.018:1$) is but slightly and evidently not significantly greater than that of form 15. But form 27 has a greater proportion of spheroidal and ellipsoid-elliptical spores, and fewer subglobose individuals. The urediniospores of form 27 are the most variable in size and shape of all the stock forms. There is a considerably lower correlation coefficient (-0.295 ± 0.062) for the length and width of the urediniospores of form 27; and the per cent of variability controlled by this correlation is only 4.49 per cent, about one-third less than in the case of form 15.

P. graminis tritici form 29.—Form 29 (Table 37) has the smallest and least variable urediniospores of the four forms cultured from multisporeous stock material. They average $28.71 \pm 0.19 \mu$ in length and $19.40 \pm 0.07 \mu$ in width, and differ significantly in both dimensions from the spores of form 15 and form 38. The difference in the mean length between the urediniospores of form 29 and form 27 is only $1.23 \pm 0.34 \mu$, and exceeds its probable error 3.62 times, which may or may not be ascribed to a mere

TABLE 37.—Correlation table for length and width of 100 urediniospores of *Puccinia graminis tritici* form 29. Spore width, subject; spore length, relative. Coefficient of correlation = -0.259 ± 0.063 ; control of variability = 3.41 per cent

		SPORE LENGTH					
		21 μ	24 μ	27 μ	30 μ	33 μ	36 μ
SPORE WIDTH	17 μ		1	1	2	1	5
	18 μ			1	6	3	11
	19 μ	1	3	13	15	6	38
	20 μ		6	11	14	4	35
	21 μ	1		3	4		8
	22 μ		1	1			2
	23 μ			1			1
		2	11	31	41	14	100

fluctuation of sampling. It is certainly does not seem to be any significant difference in the mean width of these two forms; but it may be there after all, and probably could have been demonstrated had the samples been considerably larger. In general, however, forms 29 and 27 are rather indistinguishable in size. On the other hand, the difference in size is very striking when the urediniospores of form 29 are compared with those of form 15, differing in mean length by $3.57 \pm 0.30 \mu$ and in mean width by $2.44 \pm 0.11 \mu$. In shape, the urediniospores of form 29 are very similar to those of form 15. Approximately, there are as many spheroidal, subglobose, and ellipsoidal spores in one form as in the other. Neither of the two forms possesses truly elliptical spores, i.e., those which are more than twice as long as they are broad and both forms have almost identical mean

TABLE 28.—Correlation of length and width of urediniospores of *Puccinia graminis* f. sp. *tritici* form 29. $r = 0.259$; $r_{\text{subject}} = 0.259$; $r_{\text{relative}} = 0.259$; $r_{\text{coefficient of correlation}} = 0.67$; $r_{\text{control of variability}} = 0.07$ per cent

	SPORE LENGTH					
	14 μ	17 μ	20 μ	23 μ	26 μ	30 μ
SPORE WIDTH	17 μ	1				1
	18 μ	1	1	3		2
	19 μ		1	3	10	5
	20 μ		6	10	11	5
	21 μ	1	6	10	9	4
	22 μ		3	5	1	
	23 μ			1		
	2	17	30	34	14	3
						100

length-to-width ratios, namely, $1.500 \pm 0.014:1$ in the case of form 29, and $1.518 \pm 0.015:1$ in the case of form 15. There is another decline in the correlation coefficient (-0.259 ± 0.063) between the length and width of the urediniospores, with a corresponding reduction in the variability controlled by the correlation, which in this case amounts to only 3.41 per cent.

P. graminis tritici form 38.—Form 38 (Table 38) apparently is significantly different from form 27 and form 29 in the dimensions of both diam-

eters of its urediniospores, which average $31.50 \pm 0.22 \times 20.14 \pm 0.08 \mu$, and is decidedly different from form 15 in spore width only. No truly spherical individuals were observed among the urediniospores of form 38. Some 10 per cent were spheroidal, but the majority were subglobose; about one-third were ellipsoid, and only a very few were elliptical. The ratio of length-to-width ($1.592 \pm 0.015:1$) is not significantly different from that of form 27 but definitely higher than in either form 15 or form 29. The long and short diameters of form 38 appear to be uncorrelated (-0.003 ± 0.067) but not necessarily independent. The control of variability exercised by this correlation is practically nil, viz., 0.05 per cent.

The mean length-to-width ratios, calculated by the ordinary mathematical processes in which all of the individuals concerned were taken into consideration, were compared with the quotients obtained by dividing the mean lengths established for certain populations by their corresponding mean widths. The probable errors of the quotients thus procured were computed by the formula given by Mellor (19) and quoted elsewhere in this paper. As may be gathered from table 39, no significant differences were found between the mean ratios and the ratios of the means, although such might conceivably exist and perhaps could have been demonstrated had many more cases been studied. However, it is quite possible that ratios obtained from a division of mean lengths by mean widths are, in fact, identical with the mean ratios determined by the regular method. But

TABLE 39.—Comparison of the mean length-to-width ratios of certain physiologic forms of *Puccinia graminis tritici* determined by mathematical processes and the quotients obtained from dividing the mean length of each of these forms by the respective mean width

Physiologic forms	Mean ratios of length-to-width calculated from all individuals concerned	Quotients computed by dividing each mean length by its mean width	Difference between ratios and corresponding quotients	Difference divided by respective probable error
1	1.768 ± 0.017	1.726 ± 0.013	0.042 ± 0.021	2.00
3	1.722 ± 0.018	1.714 ± 0.016	0.008 ± 0.024	0.33
9	1.656 ± 0.017	1.635 ± 0.014	0.021 ± 0.022	0.95
15	1.518 ± 0.015	1.478 ± 0.012	0.040 ± 0.019	2.11
17	1.468 ± 0.014	1.459 ± 0.011	0.009 ± 0.018	0.50
27	1.558 ± 0.018	1.533 ± 0.016	0.023 ± 0.024	0.96
29	1.500 ± 0.014	1.480 ± 0.011	0.020 ± 0.018	1.11
38	1.592 ± 0.015	1.564 ± 0.013	0.028 ± 0.020	1.40

even in such event the quotient method is not very helpful where shape of objects is under consideration. The quotient, at best, is only an abstract figure representing the arithmetical mean of all the shapes of a population; it is never an index of what the prevailing shapes or the extremes are like. These can be learned only from the frequency distribution of the shapes of all the individuals of a given random sample, from which in turn the various constants are derived.

The results given in tables 27 to 38 and in figures 25 to 30 show in a convincing way that the physiologic forms of *P. graminis tritici* differ not only in parasitic behavior but in morphology as well, and that, although the morphology of the urediniospores of a particular form may vary under varying cultural conditions, when grown under identical conditions on congenial hosts the spores have a common shape and size and other identifying characteristics. Physiologic forms, therefore, can be identified on the basis of morphology, but their parasitic behavior on certain varieties of wheat is the most satisfactory means of identification.

TABLE 40. — *Average dimensions of urediniospores of eight physiologic forms of Puccinia graminis tritici compared with the dimensions recorded for the variety tritici at large*

Physiologic forms	Arithmetic means	
	Length	Width
1	34.77 \pm 0.22	20.15 \pm 0.08
3	33.63 \pm 0.28	19.62 \pm 0.08
9	31.35 \pm 0.23	19.17 \pm 0.09
15	32.28 \pm 0.23	21.84 \pm 0.08
17	29.55 \pm 0.19	20.26 \pm 0.08
27	29.94 \pm 0.28	19.53 \pm 0.05
29	28.71 \pm 0.19	19.40 \pm 0.07
38	31.50 \pm 0.22	20.14 \pm 0.08
Average arithmetic means for the 8 physiologic forms....	31.47 \pm 0.46	20.01 \pm 0.19
Arithmetic means for variety tritici as a whole (15)....	32.40 \pm 0.19	19.79 \pm 0.06
Difference in the two means..	0.93 \pm 0.50	0.22 \pm 0.20
Difference divided by P. E....	1.86	1.10

The morphologic differences between forms of the variety *tritici* appear to be as great as, or greater than, the differences between varieties within the species *P. graminis*. It is a fact, however, (or is it a mere coincidence?) that biometrically the mean averages of the eight physiologic forms herein described are clearly not significantly different from the grand means reported for the variety *tritici* as a whole (see table 40). The means previously given by the writer (15) for the variety *P. graminis tritici* are $32.40 \pm 0.19 \times 19.79 \pm 0.06 \mu$. The averages of the means of the eight physiologic forms described above are $31.47 \pm 0.46 \times 21.01 \pm 0.19 \mu$. The difference between the two sets of figures is $0.93 \pm 0.50 \times 0.22 \pm 0.20 \mu$, a discrepancy which in all probability is within the limits of probable error due to random sampling.

There was no correlation between the differences in pathogenicity and the differences in morphometry of the physiologic forms studied.

Effect of Ecological Factors on Susceptibility of Wheat Varieties

It is common knowledge that stem rust epidemics are much more severe in some years than in others. The amount of rust which developed on the same varieties grown at different stations in any one year, or at the same station in different years, varied very considerably, even at those stations from which the same physiologic form or forms were isolated. This variation undoubtedly was due to a complexity of factors, such as the uneven quantitative distribution of the rust inoculum, the stage of development and general condition of the wheat varieties when infection first occurred, and the edaphic and climatic conditions under which the host plants were grown. Of course it also is possible that other physiologic forms were present in addition to those isolated, a fact which would have influenced the intensity of the rust epidemic at different places in different seasons.

Quantity of Inoculum

It is obvious that a sufficient number of rust spores must be present on the growing grains to give the fungus a start. The inoculum may come from infected barberries or from wind-blown, or overwintered, urediniospores, depending largely on the locality. In India, Australia, and Mexico, for instance,⁷ and even in certain parts of the Southern United States (26) the stem rust can, and evidently does, go through the winter in the uredinial stage. On the other hand, rust spores may be blown for considerable distances (28); but in the Upper Mississippi Valley of the United States barberries play a very important, if not the most important, part in starting rust epidemics (26).

⁷ LEVINE, M. N. The epidemiology of cereal rusts in general and of the black stem rust in particular. U. S. Dept. Agr., Bur. Plant Indus. Off. Cereal Invest. 78 p. 1919. [Mimeographed.]

Table 41 and figure 31 illustrate the difference in the severity of stem rust infection in two uniform rust nurseries at University Farm, St. Paul, during three consecutive years, 1921-1923. One nursery was located in the plant pathology plots (designated in the figure as "A"), where the rust epidemic was induced artificially; the other nursery was situated in the plant breeding or agronomy plots (designated in the figure as "B"), where the rust infection was allowed to take its natural course. The two nurseries were not half a mile apart and the climatic conditions therefore identical. The epidemics in both nurseries, as indicated by the greenhouse studies, evidently were due to physiologic forms capable of infecting the hard red spring wheat varieties equally well. Yet, each year the average percentage infection on these varieties was considerably higher in the pathology nursery than in the agronomy nursery.

TABLE 41.—*Estimated percentage of stem rust on nine varieties of hard red spring wheat grown in duplicate uniform rust nurseries at St. Paul, Minn., in the three years 1921-1923, inclusive*

Variety and C. I. number	Percentage of infection						
	Pathology nursery			Agronomy nursery			
	1921	1922	1923	1921	1922	1923	
Haynes Bluestem	2874	50	80	25	T	60	25
Marquis	3641	40	80	30	T	50	35
Power Fife	3697	55	85	35	25	60	25
Ruby	6047	30	85	35	T	50	20
Kitchener	4500	80	75	45	20	50	40
Red Bobs	6255	40	80	40	15	50	30
Preston	3081	45	85	45	20	65	30
Kota	5878	T	2	2	0	T	5
Prelude	4323	40	35	35	10	10	12
Average		42.2	67.4	32.4	10.0	43.9	24.7

From the data recorded in table 41, it may be seen that in 1921 the average rust infection was more than four times higher in the pathology nursery than it was in the agronomy nursery (42.2 per cent against 10.0 per cent); in 1922, the ratio was slightly better than 1.5 to 1 (67.4 per cent as compared with 43.9 per cent); and, in 1923, it was somewhat higher than 1.3 to 1 (32.4 per cent in the pathology nursery and 24.7 per cent in the agronomy nursery). These differences are consistent and significant and apparently are due to the introduction of a greater quantity of initial inoculum in the pathology nursery each year.

While it may seem self-evident that plants which have been heavily inoculated will become more heavily rusted than those less heavily inoculated, the opinion is fairly general that there usually is sufficient inoculum

abroad in any season, provided other conditions are favorable, to cause heavy infection. However, the observations here cited indicate that only abundant inoculum early in the season is conducive to heavy rust attacks. But in addition to a copious supply of inoculum, climatic conditions must be favorable at the critical time to produce a heavy rust epidemic. The cool weather and the low relative humidity in May, 1923 (see table 42 and figure 31), evidently are responsible for the rather slight excess, only 7.7 per cent, in the average infection that year in the pathology nursery over that in the agronomy nursery.

Condition of Host Plants

Freeman and Johnson (11) found that plants inoculated from the time when the heads emerged from the boot until they were in full bloom rusted much more heavily than plants inoculated either before or after this stage of development. The observations in the uniform rust nurseries have shown that there is some correlation between the severity of infection on a given susceptible variety and its stage of development, when all other conditions were equal. The nursery at Madison, Wis., was visited twice in 1922, on July 14 and August 15, respectively. On July 14 there was an abundance of leaf rust, whereas stem rust was just beginning to develop. Most of the varieties were in the soft dough stage, but Prelude was approaching maturity; Ruby, Marquis, Kahla and Peliss were in the advanced hard dough stage, and Little Club was flowering.

During the next several weeks, weather conditions were favorable for rust development. The mean temperature for the intervening month was about 70° F., and precipitation was adequate. On August 15 the green plants of Kubanka, Arnautka, and Mindum were badly rusted; the percentage of infection on some individuals was as high as 100 per cent. But the mature plants remained free from rust and the average infection on each of these varieties as a whole was estimated to be about 45 per cent. Acme, Monad, and Pentad, which had had no rust whatever on July 14, had as much as 10 and 20 per cent on individual immature plants or young shoots, and averaged 12 per cent for each of these varieties, taken as a whole. Kahla and Peliss, as well as Marquis, Ruby, Kota and Prelude, apparently escaped the rust infection, having no more than from 3 to 5 per cent rust each. But the infection on Power, Kitchener, Red Bobs, and Preston ranged from 15 to 40 per cent with an estimated average of 20 per cent.

The greatest fluctuation in infection appeared on Haynes Bluestem, ranging from 0 to 100 per cent, depending on the degree of maturity of the individual plants. The average infection on this variety also was

TABLE 42.—*Prevailing weather conditions at St. Paul, Minn., during the growing seasons of 1921, 1922, and 1923, where duplicate uniform test nurseries were operated in which, respectively, natural and artificial epidemics obtained*

Years and months	Temperature (degrees Fahrenheit)				Precipitation (in inches)			Mean relative humidity (percentages)			Weather (number of days)			Sunshine from normal		
	Maximum	Minimum	Mean	Normal	Departure	Total	Normal	Departure	7 a. m.	Noon	7 p. m.	Rainy ^a	Clear		Cloudy	Per cent of possible
1921																
May	69.1	50.6	59.8	58.2	+ 1.6	3.38	3.62	- 0.24	76.1	53.1	54.0	15	9	12	59	- 5
June	82.8	64.2	73.5	67.1	+ 6.1	4.70	4.41	+ 0.29	74.1	52.7	53.9	6	12	9	69	+ 7
July	86.8	66.6	76.7	72.1	+ 1.6	2.39	3.40	- 1.01	76.2	48.1	51.2	11	15	1	38	+ 12
August	80.0	59.9	70.0	69.5	+ 0.5	2.79	3.46	- 0.67	80.7	52.4	53.5	7	10	12	72	+ 1
1922																
May	71.5	53.5	62.5	58.2	+ 4.3	2.48	3.62	1.14	76.0	50.1	57.1	11	5	8	49	- 8
June	78.2	58.1	68.3	67.1	+ 1.2	4.61	4.11	+ 0.20	76.4	58.0	58.7	12	10	1	67	+ 9
July	78.5	59.2	68.8	72.1	- 3.3	2.32	3.40	- 1.08	80.9	56.4	50.7	7	9	5	62	- 8
August	82.5	61.6	72.0	69.4	+ 2.6	1.31	3.46	- 2.15	80.1	49.7	52.9	7	11	3	70	+ 1
1923																
May	68.9	48.1	58.6	57.9	+ 0.7	2.28	3.62	1.31	67.9	46.0	44.6	10	11	4	71	+ 17
June	80.2	59.8	70.0	67.1	+ 2.9	4.28	4.41	- 0.13	79.8	58.7	58.1	13	12	2	77	+ 15
July	85.8	61.7	75.2	72.1	+ 3.1	2.51	3.40	- 0.89	77.6	50.5	51.7	7	15	2	84	+ 14
August	76.7	57.1	66.9	69.4	- 2.5	1.92	3.16	- 1.54	78.9	50.9	52.5	10	7	5	65	- 1

^a Number of days on which 0.01 inch, or more, of precipitation occurred.

estimated to be about 45 per cent. Thirty per cent rust developed on Little Club. No rust whatever could be found on Vernal or Khapli. Form 21 alone was isolated from collections made on both visits. It would appear

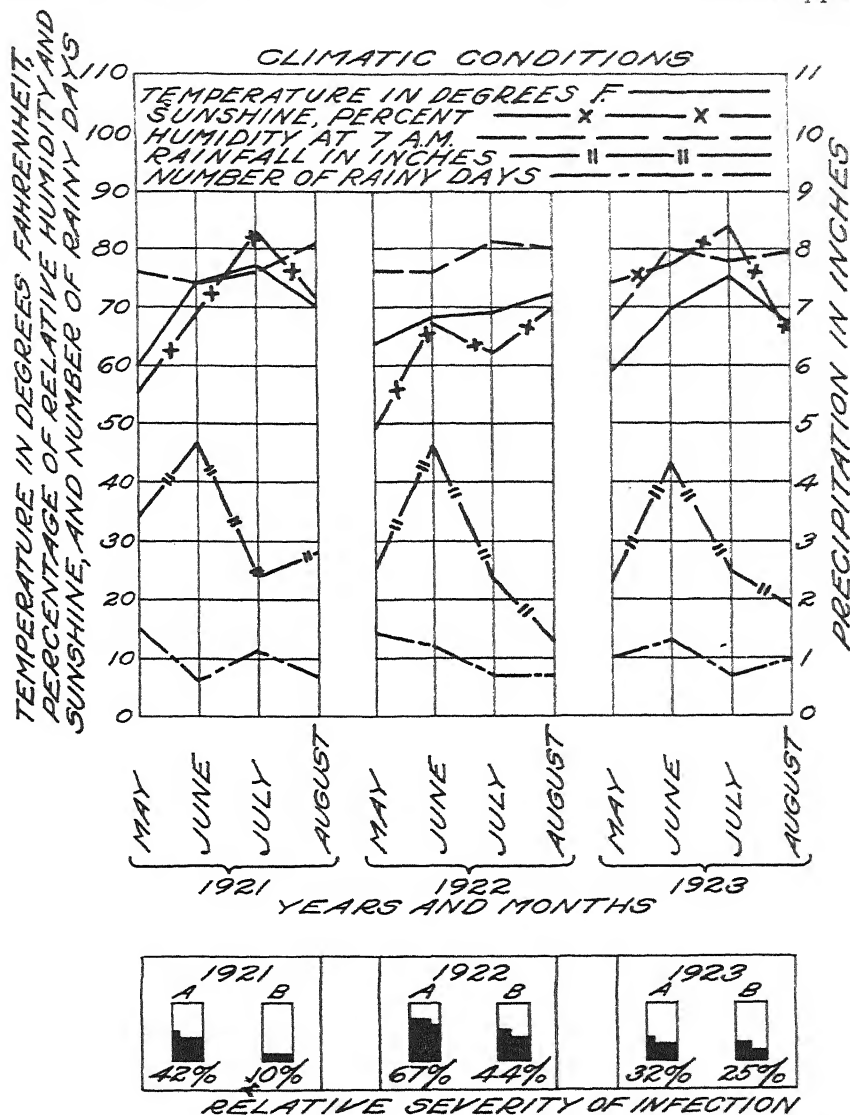


FIG. 31. Prevailing weather conditions and relative severity of stem rust infection in duplicate uniform rust nurseries, located respectively in the plant pathology (A) and plant breeding (B) plots at St. Paul, Minn., during three consecutive years, 1921-1923, inclusive.

from this and similar observations that, other conditions being equal, the degree of maturity of susceptible varieties at the time infection first takes place probably determines the severity of the rust attack on these varieties.

While the results obtained can be interpreted differently, there seems to be sufficient observational evidence to indicate that the susceptibility of plants does depend to a considerable extent on their stage of development. They seem to be quite susceptible in the seedling stage and again at about heading-out time. In the jointing stage the plants show more resistance. Whether this resistance is due to rapidity of growth, morphological characters, or to physiological processes and products within the plants can not be determined, of course, by field observations. In order to ascertain whether the apparent difference in susceptibility at different stages of host development is a real difference or only an apparent one, it would be necessary to have only one variable, namely, the age of the host plant. In the field there are many variables. It is possible that plants which appear to be resistant at different stages merely have not been exposed to infection under conditions most favorable for its development. In order to answer this question, it would be necessary to correlate the stage of development with environmental factors by means of carefully controlled greenhouse experiments.

It is well known that early-sown spring grain is less liable to suffer from stem rust than late-sown grain. This was brought out very strikingly in the duplicate sowing experiment of 1919 and 1920. The varieties were sown at an interval of two weeks, but the rust estimates were made on the same date. The difference in the amount of stem rust, as may be seen from tables 43 and 44, was particularly pronounced at Amarillo, Tex., and at Highmore, S. Dak., in 1919; and at Lincoln, Nebr., in 1920. At Amarillo all of the durum varieties in the first sowing escaped the rust attack entirely, while in the second sowing there was as much as 10 and 15 per cent infection on some of the durum varieties. Prelude was the only hard red spring variety in the first sowing which had 3 per cent stem rust; all the other varieties had a trace of stem rust or none at all. The infection on Prelude in the second sowing was estimated as 60 per cent, while some of the other varieties had 65 and 70 per cent. At Highmore, the infection on the durum varieties in the first sowing was 5 per cent or less, in the second sowing the infection on the normally susceptible varieties was as high as 65 and 80 per cent. On the hard red spring wheats in the first sowing the infection ranged from 5 to 10 per cent; in the second sowing as high as 95 and 100 per cent rust appeared. A similar situation prevailed at Lincoln in 1920.

There were only slight differences in the severity of rust attack on the varieties of the two sowings at Lincoln, Nebr., in 1919, and at St. Paul,

TABLE 43.—Percentages of stem rust on varieties of wheat grown in 1919 at seven uniform rust nurseries where duplicate sowings of the varieties were made at an interval of two weeks

Class, variety, and C. I. number	Sowings at experiment stations													
	Akron, Colo.		Anarillo, Tex.		Brookings, S. Dak.		Highmore, S. Dak.		Lincoln, Nebr.		Newell, S. Dak.		St. Paul, Minn.	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
<i>Durum</i>														
Kubanka	T	10	0	10	25	40	5	25	10	15	0	5	T	T
Kubanka	T	8	0	2	25	25	5	65	3	5	5	5	T	T
Kubanka	T	10	0	10	30	65	5	65	10	65	5	5	T	T
Kubanka	T	8	0	15	40	65	5	80	15	25	10	5	T	65
Arnautka	T	8	0	10	40	65	5	80	15	25	10	5	T	T
Arnautka	T	15	0	2	30	55	5	25	3	5	5	5	T	T
Mindum	T	15	0	T	1	0	1	5	3	2	5	5	T	T
Acme	T	0	1	1	0	1	5	2	3	5	5	0	5
Monad	0	0	0	1	0	1	5	T	0	0	5	0	0
Pentad	0	0	0	1	0	1	0	0	0	0	5	0	0
<i>Hard Red Spring</i>														
Haynes	T	35	T	65	65	100	10	100	90	100	25	40	25	50
Marquis	10	15	T	65	50	85	10	70	65	85	25	50	25	40
Marquis	3641	15	T	55	65	100	10	95	85	90	25	50	25	60
Powder	3697	20	T	70	25	100	5	20	85	65	10	65	T	25
Ruby	6047	25	T	70	65	95	10	90	90	90	25	40	20	80
Preston	3081	25	T	0	1	5	5	5	2	5	25	5	0	T
Kota	6878	15	T	3	25	100	5	15	85	65	10	25	T	T
Prelude	4323	5	10	T	40	100	10	40	65	50	10	50	15	30
Kubanka x Haynes	4788	T	10	0	50	100	10	25	40	40	25	65	5	30
Kubanka x Preston	4789	T	0	25	40	100	10	25	40	40	25	65	5	30
<i>Emmer</i>														
Emmer	3686	T	0	0	3	0	1	1	0	T	0	2	0	0
Vernal	4013	T	0	0	5	0	0	1	0	0	0	0	0	0
Khapli	4013	T	0	0	5	0	0	1	0	0	0	0	0	0
Average infection for each experiment	1.2	9.8	0.2	25.1	30.6	55.0	5.5	39.6	33.4	38.0	11.0	23.1	5.9	19.4

TABLE 44.—*Percentage of stem rust on varieties of wheat grown in 1920 at four uniform rust nurseries where duplicate sowings of the varieties were made at an interval of two weeks*

Class, variety and C. I. number	Sowings at experiment stations								
	Indian Head, Sask.		Lincoln, Nebr.		St. Paul, Minn.		Saskatoon, Sask.		
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	
<i>Triticum</i>									
Kubanka	1440	35	40	1	5	60	95	10	30
Kubanka	2094	35	35	2	10	45	100	10	30
Kubanka	4063	40	40	1	5	70	95	15	30
Arbutka	4064	35	35	1	5	95	95	10	30
Arbutka	6236	40	40	1	5	70	95	7	30
Mundum	5296	35	35	1	5	65	95	6	10
Acme	5284	15	25	1	4	40	25	3	7
Monard	3320	15	25	1	4	55	25	3	7
Pentad	3322	3	3	1	4	45	30	1	3
<i>Hard Red Spring</i>									
Haynes	2874	35	55	4	33	90	100	25	40
Marquis	3641	45	50	3	23	95	100	20	25
Power	3697	40	65	4	25	100	100	20	40
Ruby	6047	45	65	1	40	85	85	20	40
Preston	3081	45	65	4	33	95	100	25	35
Kota	5878	30	35	1	4	25	40	3	1
Prelude	4323	40	55	4	40	90	95	25	35
Kubanka x Haynes	4788	40	65	1	5	100	95	20	40
Kubanka x Preston	4789	40	65	1	4	95	100	25	35
<i>Emmer</i>									
Vernal	3686	1	1	1	2	10	15	0	0
Khapli	4013	1	1	0	0	3	5	1	1
Average infection for each experiment		30.8	40.0	1.5	12.8	66.7	74.5	12.5	23.5

Minn., in 1920. However, a comparison of the average stem rust infection on all the varieties of wheat and emmer grown at seven uniform rust nurseries in 1919, and four in 1920, showed a significant difference in the effect of the rust attack in the earlier and later sowings. Calculations by the aid of Student's method (1, 2) reveal that the odds are very great, that these differences are genuine and not due to experimental or observational error (See table 45).

TABLE 45.—*Comparison of severity of stem rust infection in uniform rust nurseries where duplicate sowings of wheat varieties were made at an interval of two weeks*

Results	Year	
	1919	1920
Number of trials	7	4
Average infection on early sowing	12.54	27.88
Average infection on later sowing	30.00	37.70
Mean difference (m. d.)	17.46	9.83
Standard deviation (σ)	9.77	1.42
z (m. d. $\div \sigma$)	1.79	6.90
Odds against chance occurrence of difference	434:1	1666:1

It is not possible to ascertain from the climatological data given in tables 46 and 47 that weather conditions were the governing factor in causing the marked difference in reaction of the varieties to rust attack in the early and late sowings. Apparently it is not a question of disease escape because of changes in the host plants incident to early maturity.

TABLE 46.—*Prevailing weather conditions during growing season of 1919 at seven uniform rust nurseries in the United States where duplicate sowings of the wheat varieties were made at an interval of two weeks*

Experiment stations and months	Temperature (degrees F.)		Precipitation (in inches)		Weather (number of days)		
	Mean	Departure from normal	Total	Departure from normal	Itainy*	Clear	Cloudy
<i>Akron, Colo.</i>							
May	55.7	1.59	-1.19	6	10	0
June	65.4	2.21	-0.37	8	17	5
July
August	73.4	0.44	-1.49	5	12	5
<i>Amarillo, Tex.</i>							
March	46.4	+1.4	1.73	+1.08	5	14	4
April	54.5	-0.1	2.56	+0.84	9	10	6
May	61.8	-2.5	2.08	-1.59	11	7	6
June	68.9	-3.1	2.94	-0.05	10	11	0
<i>Brookings, S. Dak.</i>							
May	56.4	+1.2	3.87	+0.63	11	15	7
June	68.6	+3.5	9.30	+5.64	16	9	16
July	73.6	+4.0	5.60	+3.15	11	15	1
August	68.6	+0.8	1.48	-1.33	4	15	0
<i>Highmore, S. Dak.</i>							
May	56.2	-0.4	6.63	+4.21	10	16	8
June	68.6	+0.9	1.90	-1.26	9	16	9
July	74.7	+1.8	2.65	+0.18	7	17	4
August	71.8	+0.4	0.82	-1.56	5	18	3
<i>Lincoln, Nebr.</i>							
May	60.7	-2.2	3.83	-0.42	9	8	10
June	73.0	+1.4	4.99	+0.67	13	6	10
July	82.2	+5.8	0.38	-3.45	4	21	3
August	74.8	+0.5	4.67	+0.96	8	11	10
<i>Newell, S. Dak.</i>							
May	56.8	+3.3	0.63	-2.12	6	19	7
June	70.3	+4.8	0.18	-2.44	2	14	1
July	78.0	+6.4	2.90	+0.63	7	11	2
August	73.0	+3.0	0.77	-0.51	5	26	0
<i>St. Paul, Minn.</i>							
May	57.5	-0.2	2.17	-1.49	8	11	6
June	69.1	+2.6	4.75	-0.01	13	9	12
July	72.7	+1.5	6.15	-4.07	11	14	3
August	68.2	-1.0	1.46	-1.24	10	14	2

* Number of days on which 0.01 inch, or more, of precipitation occurred.

The rust inoculum increases in geometrical progression. It is quite obvious, therefore, that those varieties which mature early are likely to escape the heaviest inoculation, while those which mature late are quite likely to be subjected to it. The difference between the amount of rust on early maturing varieties and later maturing varieties, therefore, probably is not due to the stage of development of the host plants as much as to the quantity of inoculum.

TABLE 47.—*Prevailing weather conditions during growing season of 1929 at four uniform rust nurseries in the United States and Canada where duplicate sowings of the wheat varieties were made at an interval of two weeks*

Experiment stations and months	Temperature (degrees F.)		Precipitation (in inches)		Weather (number of days)		
	Mean	Departure from normal	Total	Departure from normal	Rainy ^a	Clear ^b	Cloudy
<i>Indian Head, Sask.</i>							
May	51.0	+ 1.0	1.46	- 0.52	4	221	5
June	58.0	- 1.0	2.10	- 1.88	6	241	2
July	65.0	- 1.0	5.24	- 2.75	3	300	2
August	64.0	+ 2.0	1.42	- 0.70	7	266	3
<i>Lincoln, Nebr.</i>							
May	61.0	- 1.9	3.77	- 0.48	14	2	17
June	73.0	- 1.4	2.05	- 2.27	7	11	8
July	76.6	+ 0.2	3.80	- 0.03	10	14	3
August	71.8	- 2.5	3.69	- 0.02	7	16	7
<i>St. Paul, Minn.</i>							
May	59.0	+ 0.8	2.34	- 1.65	7	13	7
June	68.0	+ 0.6	9.64	+ 3.35	14	7	7
July	70.2	- 1.9	1.35	- 2.05	8	16	4
August	69.2	- 0.3	0.96	- 2.50	6	19	4
<i>Saskatoon, Sask.</i>							
May	52.0	+ 2.0	1.43	- 0.16	10	278	2
June	58.0	- 1.0	0.99	- 1.52	9	316	1
July	67.0	+ 5.0	2.24	- 0.30	8	378	1
August	64.0	+ 4.0	2.72	- 0.55	11	278	3

^a Number of days on which 0.01 inch, or more, of precipitation occurred.

^b For the Canadian stations the registered duration of sunshine is recorded in hours; for the American stations, the number of clear days per month are indicated.

Soil and Topography

There were two uniform rust nurseries in close proximity at Coon Creek, Minn., in 1923; one on sandy soil and the other on peat soil. The varieties on the sandy soil matured considerably earlier than the same varieties in the peat bog. In the sandy soil the plants were fully mature on July 21 when the rust notes were taken, while in the peat bog they were still in the dough stage on July 30. As will be noted from table 7, in spite

of the fact that infection was evidently caused by the same physiologic forms, there was more rust in the peat nursery than in the sand nursery. This observation would seem to confirm the conclusions of other investigators (27, 14) that conditions which are conducive to a succulent growth of the grain plants and the formation of collenchymatous tissue also are favorable to abundant production of stem rust. Of course it must be borne in mind that a difference in the soil nutrition, relative humidity, and amount of dew in the two nurseries may have been the factors which caused the differences in the severity of the rust infection.

Prevailing Weather Conditions

A careful study was made of the climatological data pertaining to the experiment stations at which uniform rust nurseries were operated^s with the object of finding a possible correlation between weather conditions and severity of rust infection. For the sake of greater accuracy it was decided to limit this study in detail to those stations where the experiment was conducted in every year of the five-year period. St. Paul, Minn., and Fargo, N. Dak., therefore were selected to represent the Prairie Area, and Mandan, and Dickinson, N. Dak., and Akron, Colo., the Great Plains Area. The estimated rust percentages on five hard red spring wheat varieties grown at each of these stations in the five years, 1919-1923, inclusive, and the calculated annual average percentages are presented in table 48. In table 49 are recorded the climatological data for these localities, covering the growing seasons of the period under consideration. A study also was made of the effect of the mean temperature and total precipitation during the last two months of the development of the wheat plant on the stem rust epidemic on three varieties of equally susceptible and uniformly maturing hard red spring wheat, grown at the different experiment stations during 89 nursery years. The results are summarized in tables 50-56, inclusive.

All the climatological data, unless otherwise stated, were obtained from the official records of the United States Weather Bureau and the Canadian Meteorological Service. In a few cases where no weather records were kept the data of the nearest meteorological station were used. The precipitation data for St. Paul, Minn., are those of University Farm; they were obtained from the Division of Soils. As the hard red spring wheat varieties, Haynes, Marquis, Power, Preston, and Kota, are practically equally susceptible under favorable conditions to all the physiologic forms

^s Thanks are due to Mr. U. G. Purssell, Associate Meteorologist, Minnesota Climatological Section, Weather Bureau, United States Department of Agriculture, for his kindness in furnishing the American weather records; and to Sir Frederic Stupart, Director, Meteorological Service, Department of Marine and Fisheries, Canada, for the liberal supply of Canadian weather records.

TABLE 48.—*Percentage of stem rust on five varieties of hard red spring wheat grown at five typical uniform nurseries in the United States in the five years, 1919-1923, inclusive*

Stations and years	Varieties and rust percentages						5-yr. average
	Hayes C.I. 2874	Marquis C.I. 2641	Power C.I. 2697	Preston C.I. 2081	Kota C.I. 3578	Group average	
<i>St. Paul, Minn.</i>							53.2
1919	50	40	60	80	0	46.0	
1920	100	100	100	100	40	88.0	
1921	50	40	55	45	T	38.0	
1922	80	80	85	85	2	66.4	
1923	25	30	35	45	2	27.4	
<i>Fargo, N. Dak.</i>							77.4
1919	100	100	100	100	10	82.0	
1920	100	65	100	100	10	75.0	
1921	95	90	100	95	5	77.0	
1922	100	98	98	95	35	85.2	
1923	75	70	80	80	35	68.0	
<i>Mandan, N. Dak.</i>							37.1
1919	40	25	37	43	T	29.0	
1920	80	65	65	75	T	57.0	
1921	10	8	6	8	0	6.4	
1922	75	40	75	70	15	55.0	
1923	40	30	50	55	15	38.0	
<i>Dickinson, N. Dak.</i>							26.6
1919	0	0	0	0	0	0.0	
1920	22	16	17	20	1	15.2	
1921	12	5	5	3	T	5.0	
1922	75	55	80	80	20	62.0	
1923	75	20	75	75	10	51.0	
<i>Akron, Colo.</i>							22.8
1919	35	15	15	25	T	18.0	
1920	65	50	60	65	15	51.0	
1921	0	T	0	0	0	0.0	
1922	2	T	5	15	7	5.8	
1923	40	40	45	50	20	39.0	

isolated, their average percentage infection was chosen as the basis for comparison.

An examination of table 49 shows that neither atmospheric temperature alone, nor total precipitation alone, whether considered for the entire growing season or for each individual month separately, can account for the difference in the severity of rust infection. But a study of figures 32-36 reveals an apparent correlation in normal years between the combined mean temperature and total precipitation in the month of June and the amount of rust at the end of the growing season. This correlation is quite pronounced at each of the five stations studied in detail, except Fargo, where other factors seem to have exerted a profound influence on the development of the rust. This question will be discussed more fully later.

TABLE 49.—*Prevailing weather conditions, during the growing season, at five typical uniform rust nurseries in the five years, 1919-1923, inclusive*

Stations and years	Climatological data											
	Mean temperature, ° F.				Total precipitation in.				Number rainy days ^a			
	May	June	July	Aug.	May	June	July	Aug.	May	June	July	Aug.
<i>St. Paul, Minn.</i>												
1919	57.5	69.1	72.7	68.2	2.17	4.75	6.15	1.46	8	12	11	10
1920	59.0	68.0	70.2	69.2	2.34	9.64	1.35	0.96	7	14	8	6
1921	59.8	73.5	76.7	70.0	4.10	3.19	4.27	1.05	15	6	11	7
1922	62.5	68.3	68.8	72.0	2.86	6.76	1.73	1.55	14	12	7	7
1923	58.6	70.0	75.2	66.9	3.05	4.95	2.90	1.90	10	13	7	10
<i>Fargo, N. Dak.</i>												
1919	57.0	69.0	71.9	68.2	4.01	2.50	5.56	2.83	12	10	8	8
1920	55.6	64.8	68.9	68.4	2.53	5.89	2.92	1.33	11	14	8	7
1921	56.6	70.4	72.8	68.2	2.56	1.40	3.08	2.94	8	7	8	11
1922	59.5	65.6	67.3	71.4	3.94	2.93	1.65	0.74	13	8	8	7
1923	55.8	68.1	72.5	63.9	1.85	6.73	2.34	2.83	7	11	7	8
<i>Mandan, N. Dak.</i>												
1919	55.8	69.0	74.2	71.0	3.90	1.17	0.85	1.22	11	6	7	7
1920	56.4	65.4	71.4	70.6	1.72	1.63	2.68	1.80	8	11	7	5
1921	60.4	72.2	78.1	70.0	5.13	3.52	2.46	3.97	11	6	9	4
1922	57.8	66.0	66.6	72.4	2.05	3.43	3.17	0.31	17	11	10	5
1923	56.4	67.2	73.4	64.8	1.82	1.94	4.12	1.15	6	13	12	5
<i>Dickinson, N. Dak.</i>												
1919	54.6	69.9	73.8	69.8	2.49	0.52	0.53	0.59	11	4	5	6
1920	52.5	61.6	67.3	68.0	1.39	4.32	2.76	2.35	13	12	8	7
1921	51.0	69.2	71.0	66.7	1.78	3.09	1.01	2.73	10	8	7	5
1922	54.1	63.3	64.4	69.2	1.97	6.57	1.92	0.69	14	14	14	6
1923	52.4	65.3	70.0	63.1	1.46	4.49	4.67	0.82	9	8	10	9
<i>Akron, Colo.</i>												
1919	55.7	65.4	...	73.4	1.59	2.21	...	0.44	6	8	..	5
1920	64.1	71.4	67.0	...	4.89	4.72	1.45	..	11	6	8
1921	56.6	67.6	74.3	...	0.50	1.06	2.25	...	12	12	4	..
1922	56.5	68.4	71.6	75.0	3.69	1.43	3.24	1.24	11	6	12	9
1923	54.4	...	73.2	69.6	4.94	...	3.02	0.78	12	..	11	7

^a Number of days on which 0.01 inch, or more, of precipitation occurred.

At St. Paul, Minn., (See table 49 and figure 32), in 1919, the total precipitation for June (4.75 inches) was a little more than one inch below the five-year average; the mean temperature (69.1° F.) was more than two and one-half degrees above normal. The rust infection on the five common wheat varieties was 46 per cent, or about 7 per cent lower than the average for the five years. In 1920, the precipitation in June (9.64 inches) was almost four inches above the average, while the mean temperature (68° F.) was only about half a degree above normal. There was an average of 88 per cent stem rust on the common wheats that year, or 35 per cent more than the five-year average. In 1921, very high temperature (73.5° F.) and very low precipitation (3.19 inches) prevailed in June and the rust infection was almost 15 per cent less than the five-year average. In 1922,

the mean temperature in June was only 1.2° above normal (65.3° F.) and the total precipitation was about one inch above the five-year average. The average rust infection was 66.4 per cent, or about 13 per cent more than the five-year average. In 1923, below average precipitation and rather high temperature in June were correlated with a fairly meager quantity of rust 27.4 per cent. Similar correlations, as may be seen from figures 34 and 35, respectively, are apparent at Mandan and Dickinson, N. Dak. In the case of Akron, Colo., the correlation is not so striking, although it is fairly definite. This may be due in part to the fact that the climatological data for this station are not quite complete, and estimated figures were substituted for the missing data.

At Fargo, N. Dak., we find no such correlations as we have found in the case of the other four stations. The heaviest average infection, 85.2 per cent, on the five common wheat varieties in the Fargo nursery occurred in 1922. The June temperature (65.6° F.) was 1.3° above normal and the precipitation was low (2.93 inches), being one inch below normal. In 1919, also there was a very heavy stem rust epidemic, averaging 82 per cent. This year the June conditions seemed even less favorable for rust development, namely, fairly high temperature (69° F.), 4.7° above normal, and fairly low precipitation (2.50 inches), about 1.5 inches below normal. In 1923, the temperature and rainfall conditions seemed to be very favorable for the development of stem rust, but this was the only year in which there was the least amount of rust at this station, namely, 68 per cent. In that year precipitation in June was very high (6.73 inches), about three inches above normal, and moderate temperature prevail (68.1° F.). There was 9 per cent more rust in 1921 than in 1923, although the conditions did not appear at all so favorable. The mean temperature in June was rather high for Fargo (70.4° F.), over 6° above normal, and the precipitation (1.40 inches) was almost 3 inches below normal. June of 1920 was much cooler than that of 1921 (64.8° F.) and the rainfall was considerably heavier (5.89 inches), yet the average amount of rust on the five common wheats was virtually the same in both years.

There is another interesting point about the Fargo situation. It will be observed from table 48 that the highest five-year average rust infection was at Fargo. The rust epidemic on the five hard red spring varieties was worst at Fargo each year of the investigation except 1920, when the infection at St. Paul exceeded that of Fargo by an average of 13 per cent. From the data at hand, it is difficult to ascertain definitely what other factors have contributed to this apparent anomaly. Of course wheat matures somewhat later at Fargo than at St. Paul, and the July conditions perhaps should be considered in preference to those of June. But this also

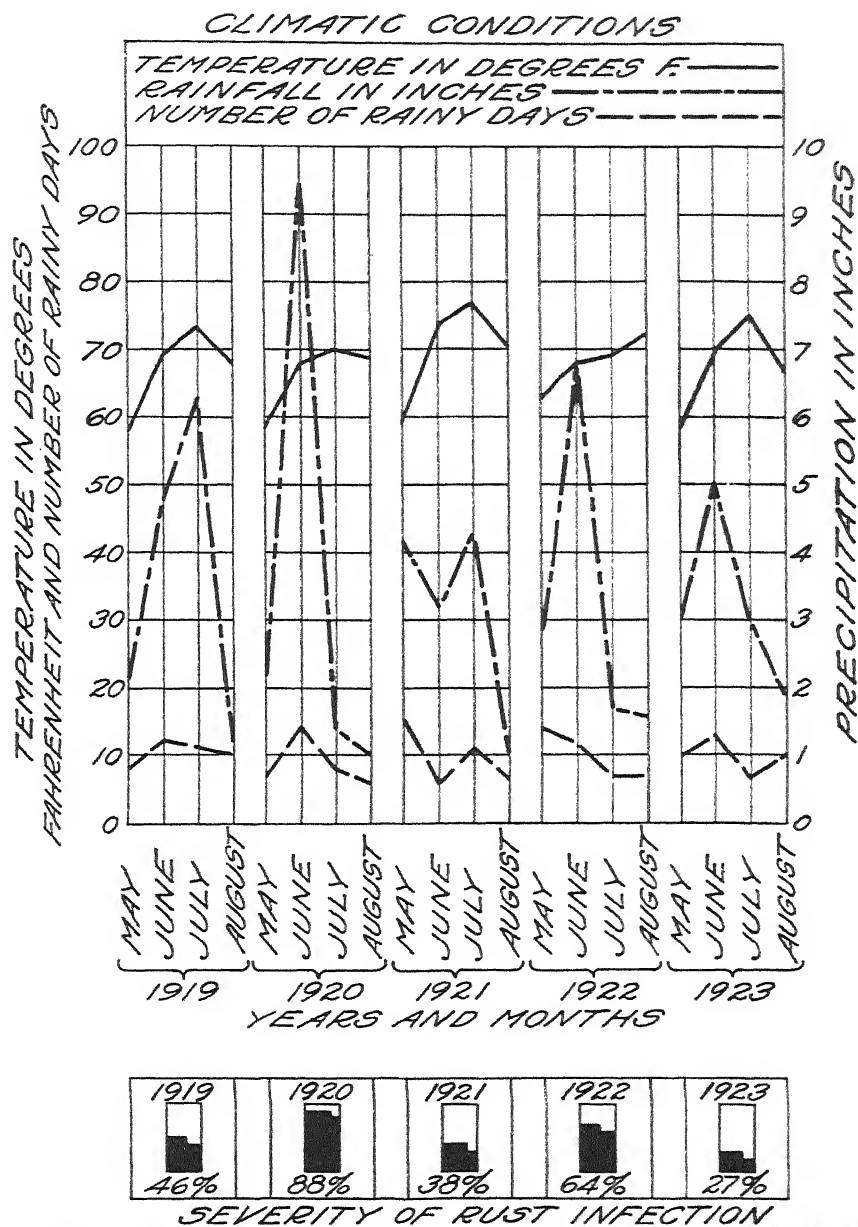


FIG. 32. Relation of temperature, rainfall and number of days on which 0.01 inch, or more, of precipitation occurred, to the severity of stem rust infection on five varieties of hard red spring wheat, grown in the uniform rust nursery at St. Paul, Minn., in the five years, 1919-1923, inclusive.

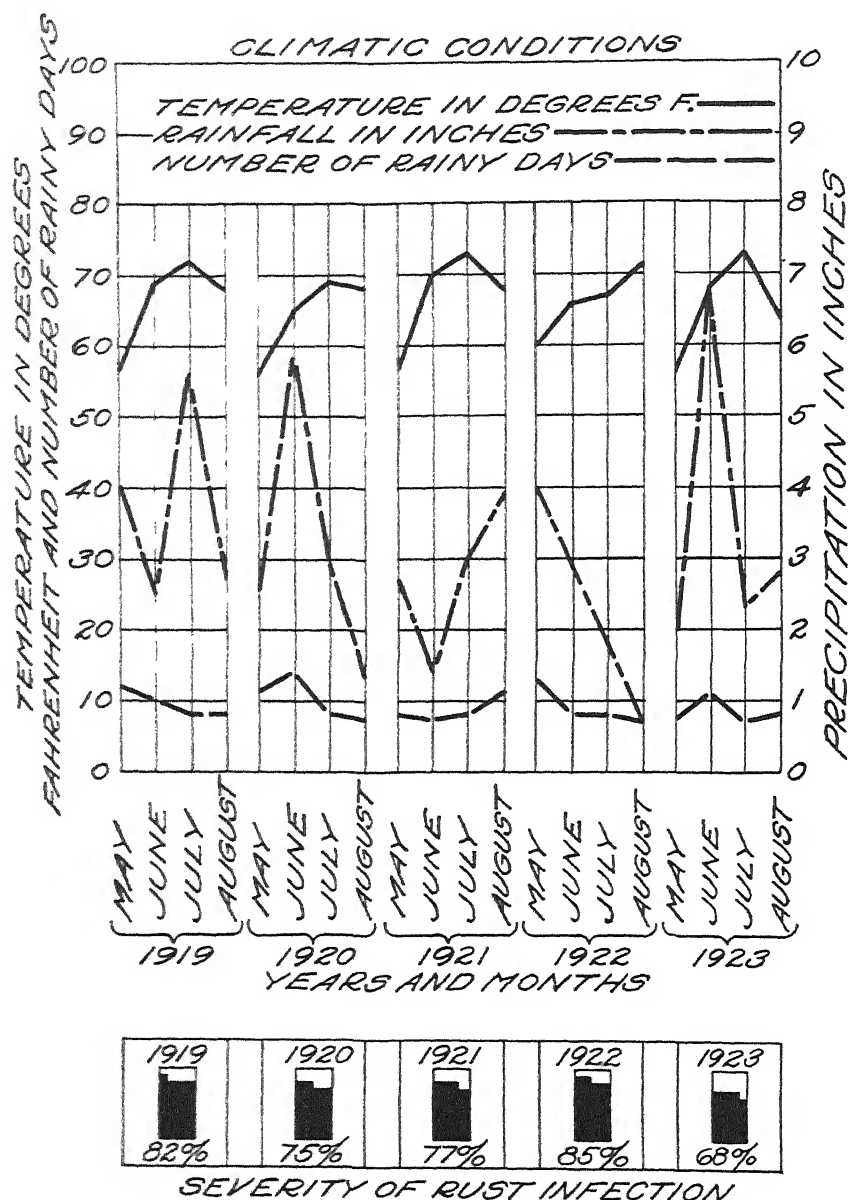


FIG. 34. Relation of temperature, rainfall, and number of days on which 0.01 inch, or more of precipitation occurred, to the severity of stem rust infection on five varieties of hard red spring wheat, grown in the uniform rust nursery at Fargo, N. Dak., in the five years, 1919-1923, inclusive.

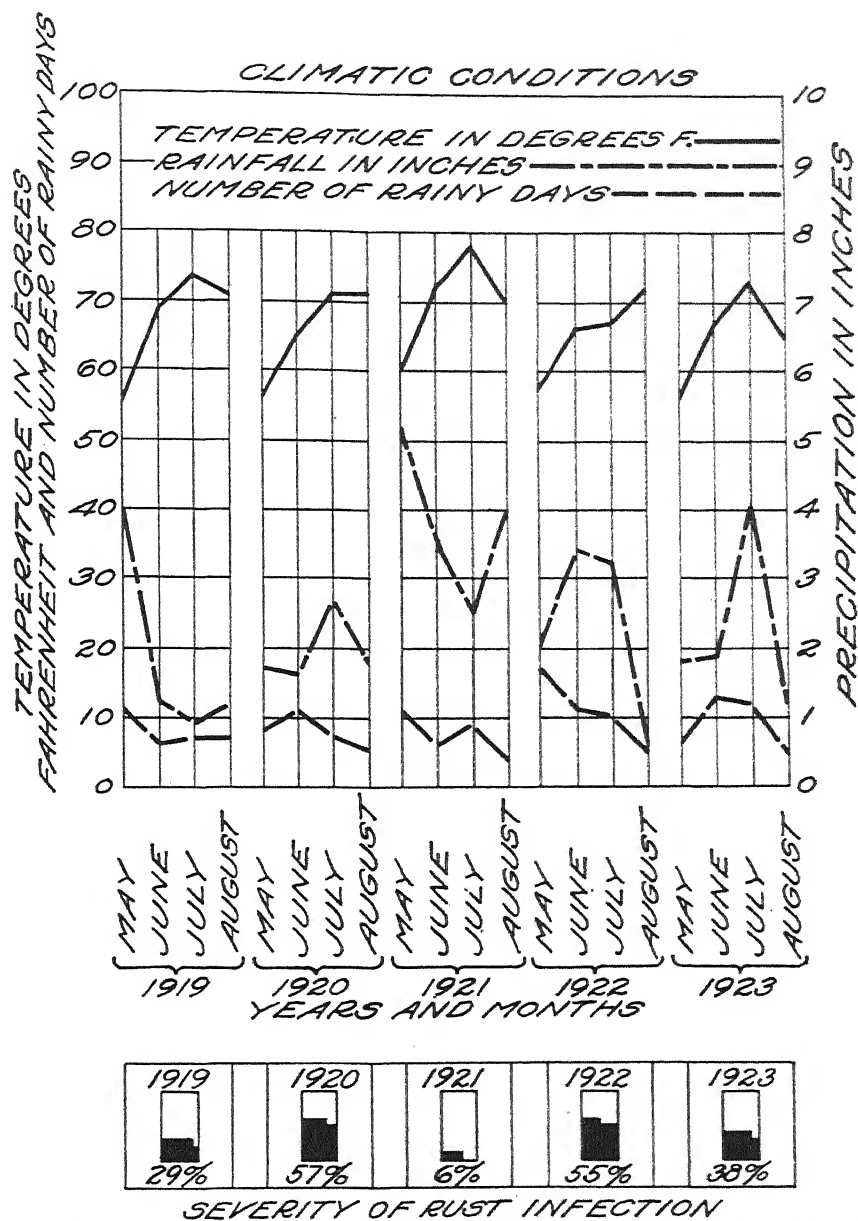


FIG. 34. Relation of temperature, rainfall, and number of days on which 0.01 inch, or more, of precipitation occurred, to the severity of stem rust infection on five varieties of hard red spring wheat, grown in the uniform rust nursery at Mandan, N. Dak., in the five years, 1919-1923, inclusive.

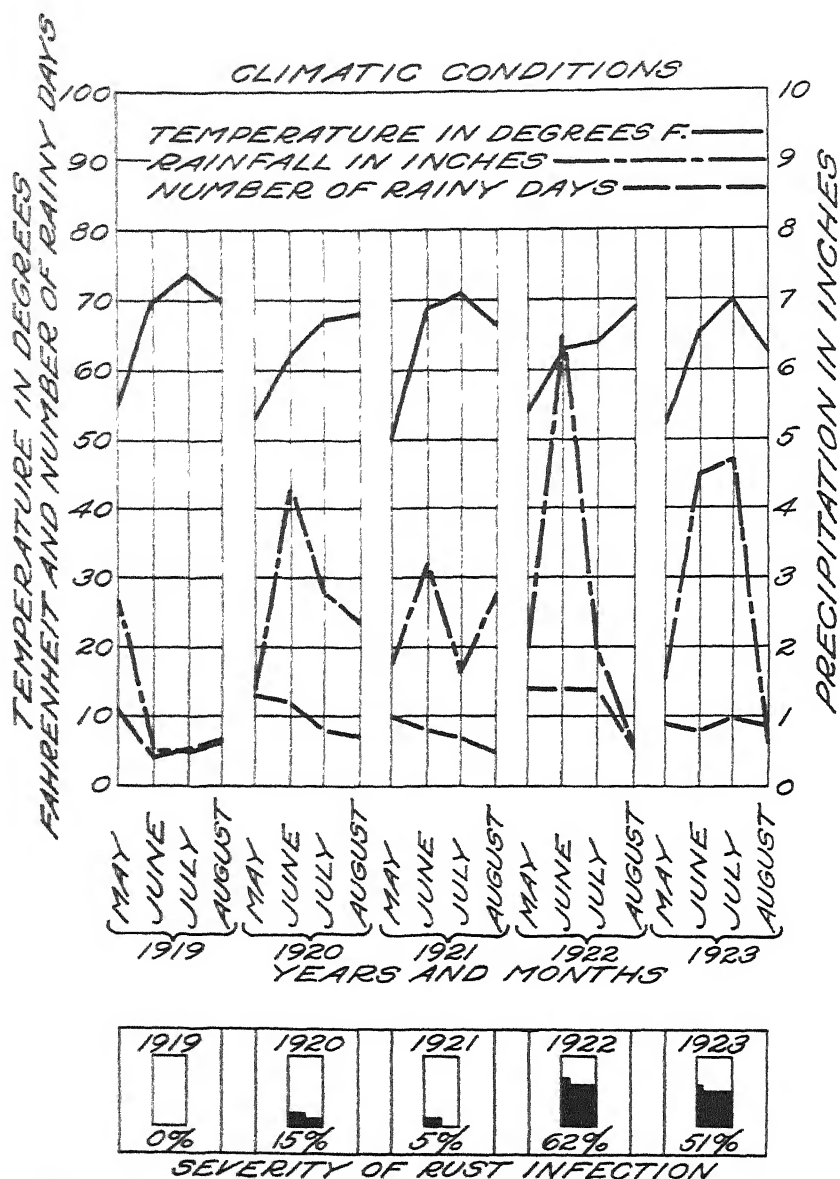


FIG. 35. Relation of temperature, rainfall, and number of days on which 0.01 inch, or more, of precipitation occurred, to the severity of stem rust infection on five varieties of hard red spring wheat, grown in the uniform rust nursery at Dickinson, N. Dak., in the five years, 1919-1923, inclusive.

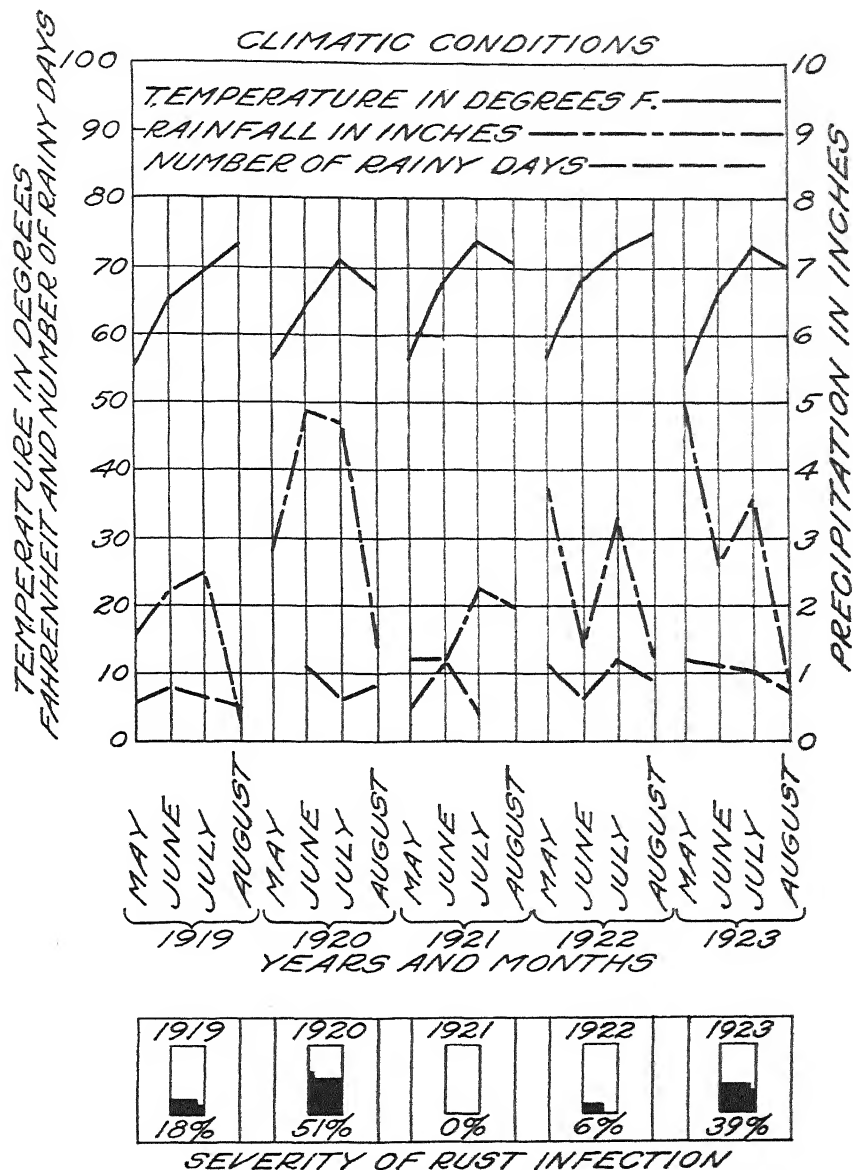


FIG. 36. Relation of temperature, rainfall, and number of days on which 0.01 inch, or more, of precipitation occurred, to the severity of stem rust infection on five varieties of hard red spring wheat, grown in the uniform rust nursery at Akron, Colo., in the five years, 1919-1923, inclusive.

is true of the other western stations, and there June seems to be the critical month. Moreover, a consideration of July does not improve the situation at Fargo. It would thus appear that the monthly mean temperatures and total rainfall during the growing season can not always be considered as the sole contributors to, and safe indices of, the severity of stem rust infection in a given locality in a given season.

Leaving a situation like Fargo out of consideration, it may be stated on the basis of the results just discussed that, in a general way, in addition to the presence of sufficient initial inoculum, a mean temperature in June fluctuating between 63° and 70° F., combined with a total precipitation in that month ranging from about 2.5 to 9.5 inches, adequately distributed, probably are the most favorable climatic conditions for the development of stem rust epidemics on susceptible varieties in certain localities in the hard red spring wheat region. Neither at Fargo, nor at any of the other four stations, was there observed any consistent relationship between the number of rainy days in any given month and the amount of stem rust in a given season.

In addition to the more detailed study of the possible correlation between temperature and rainfall on the one hand and stem rust infection on the other, it was deemed advisable to determine the effect of the mean temperature and total precipitation of the last two months of the growing season on the development of stem rust throughout the experimental territory during the whole five-year period. To obviate complications, incident to differences in varietal susceptibility, time of maturity, and pathogenicity of physiologic forms, three varieties of hard red spring wheat (Haynes, Power, and Preston) were chosen because of their similar growth period and uniform rust susceptibility; also, only such nurseries and experimental years were taken into consideration from and in which physiologic forms were isolated that under normal conditions attacked very heavily the three varieties in question.

The various uniform rust nurseries are listed in tables 50 to 52, according to the time of maturing of the three wheat varieties. In each of these tables are recorded the percentages of rust on each variety, each year, at each locality. Also the average infection for the three varieties is given. The corresponding monthly and bi-monthly mean temperatures and total precipitations also are included. From these data, the correlations between 264 varietal units and the climatic conditions in 89 nursery years were determined by the accepted methods.²

² Grateful acknowledgment is made to Dr. H. K. Hayes, Professor of Plant Breeding, University of Minnesota, and to Dr. O. K. Tedin, formerly of the Institute of Genetics, University of Lund, Sweden, recently Fellow, International Education Board, and situated at the University of Minnesota, for their kind assistance in computing the data.

TABLE 50.—*Estimated stem rust infection on three varieties of hard red spring wheat of similar susceptibility and maturity, and prevailing climatic conditions during May and June, at several experiment stations in the Central West and South West, during the five-year period, 1919-1923, inclusive*

Years and stations	Rust infection				Climatological data					
	Haynes C.I. 2874	Power C.I. 3697	Preston C.I. 3081	Ave.	Mean temperature (° F.)			Total precipitation (in.)		
					May	June	Ave.	May	June	Sum
1919										
Ames, Iowa . . .	75	65	65	68.3	57.4	72.0	64.7	2.34	2.88	5.22
Lincoln, Nebr. .	100	90	90	93.3	60.7	73.0	66.8	3.83	4.99	8.82
Manhattan, Kans.	90	80	90	86.7	62.8	73.7	68.3	3.13	4.01	8.04
Anarillo, Tex. .	65	55	70	63.3	61.8	68.9	65.4	2.08	2.94	5.02
Akron, Colo. . . .	35	15	25	25.0	55.7	65.4	60.6	1.59	2.21	3.80
1920										
Lincoln, Nebr. .	33	25	33	30.3	61.0	73.0	67.0	3.77	2.05	5.82
Akron, Colo. . . .	65	60	65	63.3	...	64.1	60.0 ^a	...	4.89	7.69 ^a
1921										
Madison, Wis. .	90	70	80	80.0	60.4	72.2	66.3	5.13	3.52	8.65
Ames, Iowa . . .	40	30	35	35.0	63.7	74.6	69.2	2.81	4.41	7.22
Akron, Colo. . . .	0	0	0	0.0	56.6	67.6	62.1	0.50	1.06	1.56
1922										
Coon Creek, Minn.	25	..	40	32.5	63.2	69.4	66.3	1.99	2.74	4.73
Waseca, Minn. .	95	95	75	88.3	62.6	70.4	66.5	3.46	2.00	5.46
Madison, Wis. .	45	20	20	28.3	63.6	68.6	66.1	4.16	3.17	7.33
Ames, Iowa . . .	35	30	45	36.7	63.0	72.8	67.9	8.36	0.87	9.23
Lincoln, Nebr. .	60	40	25	41.7	64.0	75.8	69.9	2.73	2.95	5.68
Akron, Colo. . . .	2	5	15	7.3	56.5	68.4	62.5	3.69	1.43	5.12
1923										
Coon Creek, Minn.	25	25	25	25.0	59.6	71.2	65.4	3.95	6.13	10.08
Waseca, Minn. .	35	45	50	43.3	58.6	70.1	64.4	1.52	6.15	7.67
Madison, Wis. .	40	50	70	53.3	57.0	71.2	64.1	1.90	3.05	4.95
Ames, Iowa . . .	75	75	75	75.0	59.8	71.0	65.4	2.49	5.29	7.78
Lincoln, Nebr. .	12	15	10	12.3	60.4	71.8	66.1	2.54	5.76	8.30
Akron, Colo. . . .	40	45	50	45.0	54.4	...	60.2 ^a	4.94	...	7.54 ^a

^a Normal for missing months included in estimated average.

There seems to be a definite correlation between the mean temperature of the last two months of the growing season and the severity of stem rust infection on susceptible varieties of wheat. Likewise, the total rainfall for these two months can be positively correlated with the intensity of the rust epidemics. However, the correlation coefficients in either case are rather low, $+0.283 \pm 0.038$ and $+0.336 \pm 0.037$, respectively; and the reductions in variability, 4.09 per cent and 5.81 per cent, respectively, are correspondingly small (Tables 53 and 54). There appears to be a rather insignificant positive correlation between the mean temperature and total precipitation of the period in question, namely $+0.074 \pm 0.071$ (Table 55). This probably accounts for the slight reduction in the coefficients of the partial correlations of the amount of rust and mean temperature, with

TABLE 51. — *Estimated stem rust infection on three varieties of hard red spring wheat of similar susceptibility and maturity, and prevailing climatic conditions during June and July at several experiment stations in Minnesota, North Dakota, and South Dakota during the five-year period, 1919-1923, inclusive*

Years and stations	Ru-t infection				Climatological data					
	Haynes	Power	Preston	Ave.	Mean temperature (° F.)			Total precipitation (in.)		
	C.I. 2874	C.I. 3697	C.I. 3081		June	July	Ave.	June	July	Sum
1919										
St. Paul, Minn. .	50	60	50	66.7	69.1	72.7	70.9	4.75	6.15	10.90
Fargo, N. Dak. .	100	100	100	100.0	69.0	71.9	70.5	2.50	3.56	5.06
Mandan, N. Dak.	40	37	43	40.0	69.0	74.2	71.6	1.17	0.85	2.02
Dickinson, N. Dak.	0	0	0	0.0	69.9	73.8	71.9	0.52	0.53	1.05
Brookings, S. Dak.	100	100	95	98.3	68.6	73.6	71.1	9.30	5.60	14.90
Higmore, S. Dak.	100	95	90	95.0	65.6	74.7	71.7	1.90	2.65	4.55
Nevelle S. Dak. .	40	40	40	40.0	70.3	78.0	74.2	0.18	2.90	3.08
1920										
St. Paul, Minn. .	100	100	100	100.0	68.0	70.2	69.1	9.64	1.35	10.99
Fargo, N. Dak. .	100	100	100	100.0	64.8	68.9	66.9	5.89	2.92	8.81
Mandan, N. Dak.	80	65	75	73.3	65.4	71.4	68.4	1.63	2.68	4.31
Dickinson, N. Dak.	22	17	20	20.0	61.6	67.3	64.4	4.22	2.76	7.08
1921										
St. Paul, Minn. .	50	55	45	50.0	73.5	76.7	75.1	3.19	4.27	7.46
Crookston, Minn.	85	85	90	86.7	69.8	73.6	71.7	2.69	3.02	5.71
Morris, Minn. .	75	15	40	43.3	70.0	74.1	72.1	2.83	3.58	6.41
Fargo, N. Dak. .	95	100	95	93.3	70.4	72.8	71.6	1.40	3.08	4.48
Langdon, N. Dak.	25	25	25	25.0	64.5	68.0	66.3	2.55	4.19	6.74
Edgeley, N. Dak.	70	75	75	73.3	68.0	72.7	70.4	5.76	2.61	8.37
Mandan, N. Dak.	10	6	8	8.0	70.8	74.6	72.7	1.35	3.38	4.73
Dickinson, N. Dak.	12	5	3	6.7	69.2	71.0	70.1	3.09	1.61	4.70
Brookings, S. Dak.	55	55	40	50.0	71.4	74.0	72.7	0.85	3.44	4.29
1922										
St. Paul, Minn. .	80	85	85	83.3	68.3	68.8	68.6	6.76	1.73	8.49
Crookston, Minn.	90	90	90	90.0	66.6	68.1	67.4	1.77	3.45	5.22
Morris, Minn. .	60	50	75	61.7	68.0	68.4	68.2	0.99	3.17	4.16
Fargo, N. Dak. .	100	98	95	97.7	65.6	67.3	66.5	2.93	1.65	4.58
Langdon, N. Dak.	50	45	65	53.3	61.6	63.3	62.5	4.12	2.96	7.08
Edgeley, N. Dak.	35	40	25	33.3	65.5	66.2	65.9	2.44	1.84	3.78
Mandan, N. Dak.	75	75	70	73.3	66.0	66.6	66.3	3.43	3.17	6.60
Dickinson, N. Dak.	75	80	80	78.3	63.3	64.4	63.9	0.57	1.92	2.49
Brookings, S. Dak.	95	75	90	86.7	69.5	68.3	68.9	3.75	2.81	6.56
Redfield, S. Dak.	80	80	55	71.7	70.1	70.7	70.4	2.67	0.80	3.47
1923										
St. Paul, Minn. .	25	35	45	35.0	70.0	75.2	72.6	4.95	2.90	7.85
Crookston, Minn.	70	60	60	63.3	69.7	72.4	71.1	3.34	4.77	8.11
Morris, Minn. .	95	95	95	95.0	69.4	74.1	71.8	3.50	3.42	6.92
Fargo, N. Dak. .	75	80	80	78.3	68.1	72.5	70.3	6.73	2.34	9.07
Langdon, N. Dak.	90	90	98	92.7	65.4	70.2	67.6	1.98	0.70	2.68
Edgeley, N. Dak.	90	85	85	86.7	65.5	71.4	68.5	6.43	3.10	9.53
Mandan, N. Dak.	40	50	55	48.3	67.2	73.4	70.3	1.94	4.12	6.06
Dickinson, N. Dak.	75	75	75	75.0	65.3	70.0	67.7	4.49	4.67	9.16
Brookings, S. Dak.	70	70	75	71.7	68.8	74.1	71.5	5.74	1.94	7.68
Redfield, S. Dak.	55	55	60	56.7	69.4	75.0	72.2	5.33	2.13	7.46

TABLE 52.—*Estimated stem rust infection on three varieties of hard red spring wheat of similar susceptibility and maturity, and prevailing climatic conditions during July and August, at several experiment stations in the Northern and Western United States and in Western Canada, during the five-year period, 1919-1923, inclusive*

Years and stations	Rust infection				Climatological data					
	Haynes C.I. 2874	Power C.I. 3697	Preston C.I. 3081	Ave.	Mean temperature (° F.)			Total precipitation (in.)		
					July	Aug.	Ave.	July	Aug.	Sum
1919										
Archer, Wyo. ...	65	65	65	65.0	69.6	68.4	69.0	2.75	1.20	3.95
1920										
Archer, Wyo. ...	50	50	50	50.0	65.8	63.4	64.6	1.25	1.27	2.52
Morden, Man. ...	50	50	65	55.0	69.0	69.0	69.0	1.74	0.59	2.33
Winnipeg, Man. .	65	50	75	63.3	66.0	70.0	68.0	0.76	1.70	2.46
Brandon, Man. .	65	50	65	60.0	65.0	67.0	66.0	2.15	4.34	6.49
Indian Head, Sask.	55	65	65	61.7	65.0	64.0	64.5	5.24	1.42	6.66
Saskatoon, Sask. .	40	40	35	38.3	67.0	64.0	65.5	2.24	2.72	4.96
Rosthern, Sask. .	75	40	35	50.0	66.0	65.0	65.5	2.53	1.07	4.20
Scott, Sask.	15	8	20	14.3	64.0	64.0	64.0	3.74	2.37	6.11
Edmonton, Alta. .	25	15	25	21.7	65.0	59.0	62.0	2.33	1.97	4.30
1921										
Duluth, Minn. . .	50	..	55	52.5	70.8	63.6	67.2	5.41	2.84	8.25
Morden, Man. ...	80	75	80	78.3	70.0	64.0	67.0	6.60	3.40	10.00
Winnipeg, Man. .	60	70	85	71.7	70.0	64.0	67.0	3.71	2.46	6.17
Brandon, Man. . .	50	40	40	43.3	69.0	64.0	66.5	0.61	2.60	3.21
Indian Head, Sask.	80	10	35	41.7	65.0	61.0	63.0	3.78	1.13	4.91
Saskatoon, Sask. .	5	30	50	28.3	66.0	58.0	62.0	3.04	0.31	3.35
Rosthern, Sask. .	80	80	75	78.3	65.0	61.0	63.0	4.91	0.64	5.55
Scott, Sask.	0	T	T	T	63.0	60.0	61.5	1.65	0.56	2.21
Vermilion, Alta. .	0	0	0	0.0	61.0	57.0	59.0	4.26	2.97	7.23
Edmonton, Alta. .	0	0	0	0.0	61.0	57.0	59.0	3.65	1.56	5.21
Lacombe, Alta. . .	0	0	0	0.0	61.0	58.0	59.5	3.28	0.98	4.26
1922										
Chatham, Mich. .	15	25	20	20.0	62.8	63.6	63.2	5.14	1.22	6.36
Duluth, Minn. ...	68	..	60	64.0	63.6	64.2	63.9	2.30	2.01	4.31
Archer, Wyo. ...	0	T	T	T	65.5	69.0	67.3	1.81	2.35	4.16
1923										
Chatham, Mich. .	30	20	40	30.0	64.2	58.0	61.1	5.56	2.75	8.61
Duluth, Minn. ...	65	90	60	71.7	64.6	60.3	62.5	5.40	1.76	7.16
Archer, Wyo. ...	35	50	50	45.0	67.5	63.7	65.6	1.62	2.92	4.54

rainfall constant, on the one hand, and the severity of infection and total precipitation with temperature constant, on the other, as compared with those of the independent correlations (See table 56).

Figure 37 illustrates the combined effect of mean temperature and total precipitation on the development of stem rust. Here the average infection on the three susceptible wheat varieties was correlated with the weather conditions in the 89 nursery years. No infection whatever occurred where the total rainfall was below 2 inches or where the temperature was less

TABLE 53.—*Correlation between stem rust infection on three susceptible varieties of hard red spring wheat and the mean temperature during the last two months of wheat growth. Mean temperature, subject; rust infection, relative. Coefficient of correlation = $+0.283 \pm 0.038$; control of variability = 4.09 per cent*

	RUST INFECTION						
	0%	20%	40%	60%	80%	100%	
TEMPERATURE (FAHRENHEIT)							
60°	9	3	5	4			21
63°	10	13	6	9	8	1	47
66°	3	14	14	19	17	15	82
69°	2	2	8	15	22	11	60
72°	5	3	7	9	8	16	48
75°			4	2			6
	20	35	44	58	55	43	264

TABLE 54.—*Correlation between stem rust infection on three susceptible varieties of hard red spring wheat and the total precipitation during the last two months of wheat growth. Total precipitation, subject; rust infection, relative. Coefficient of correlation = $+0.336 \pm 0.037$; control of variability = 5.81 per cent*

	RUST INFECTION						
	0%	20%	40%	60%	80%	100%	
PRECIPITATION (INCHES)							
0"	3						3
3"	13	6	15	21	7	6	68
6"	13	21	18	26	17	18	113
9"		8	11	9	30	13	71
12"				2	1	3	6
15"						3	3
	29	35	44	58	55	43	264

than 60° F. Barring one exception, not more than about 40 per cent rust appeared at or below 62° F. Ninety to one-hundred per cent stem rust was found only at the mean temperature range of 66 to 72° F., coupled with a total rainfall exceeding 2.5 inches. The multiple correlation between these two weather conditions and the severity of the stem rust infection was + 0.4239, indicating a reduction of variability of 9.43 per cent (Table 56). These results would justify the conclusion that in the final analysis weather conditions of the last two months of the growing season play only a minor, even if very important, part in the intenseness of stem rust epidemics, for it is held by statisticians that, if all essential factors have been considered, the multiple correlation coefficient should be 0.9, or above.

Sunshine, as indicated by the number of clear days per month, seems to be of no value in so far as determining its effect on the severity of infection goes. All other conditions being favorable, the normal light intensity during the growing season seems to be quite sufficient for the best development of the rust fungus. A proper amount of dew at the critical time after all may be the decisive factor in infection. This is a matter that deserves due consideration and careful study. The possibility that undetected virulent physiologic forms are responsible for the intensity of an infection is certainly not exhausted.

Aecial Host

Last but not least the rôle the common barberry is likely to play in the severity of a rust epidemic must be given serious consideration. Bringing

TABLE 55.—*Correlation between mean temperature and total precipitation during the last two months of wheat development in eighty-nine nursery years. Mean temperature, subject; total precipitation, relative. Coefficient of correlation = + 0.074 ± 0.071; control of variability = 0.28 per cent*

	PRECIPITATION (INCHES)					
	0"	3"	6"	9"	12"	15"
TEMPERATURE (FAHRENHEIT)						
60°		2	2	3		7
63°		5	9	2		16
66°		5	15	8	1	29
69°		7	5	6	2	20
72°	1	2	7	3	1	15
75°		1	1			2
	1	22	39	22	4	1
						89

TABLE 56.—*Correlation coefficients and regression values for stem rust infection on three varieties of hard red spring wheat, equally susceptible to stem rust and maturing at about the same time, and the mean temperature and total precipitation during the last two months of wheat development*

Series of variables		Coefficients of correlation	Regression values
Symbols ^a	Combinations		
<i>Correlations</i>			
r12	Rust infection and mean temperature.....	+ 0.2828
r13	Rust infection and total precipitation.....	+ 0.3357
r23	Mean temperature and total precipitation....	+ 0.0740
<i>Regression values</i>			
<i>Total precipitation, constant</i>			
b12.3	Rust infection and mean temperature....	+ 0.2594
b21.3	Mean temperature and rust infection.....	+ 0.2907
<i>Mean temperature, constant</i>			
b13.2	Rust infection and total precipitation....	+ 0.3165
b31.2	Total precipitation and rust infection.....	+ 0.3422
<i>Partial correlations</i>			
<i>Total precipitation, constant</i>			
r12.3	Rust infection and mean temperature.....	+ 0.2746
<i>Mean temperature, constant</i>			
r13.2	Rust infection and total precipitation.....	+ 0.3291
<i>Multiple correlation</i>			
B1.23	Combined effect of mean temperature and total precipitation on stem rust infection..	+ 0.4239

^a r = correlation coefficient; b = regression value; R = coefficient of multiple correlation; 1 = rust infection; 2 = mean temperature; 3 = total precipitation.

up the Fargo question again, we note that according to the findings of Melhus, Durrell and Kirby (18) the weather conditions in May of 1922 and 1919 were extremely favorable for the germination of teliospores and sporidia. We find furthermore that the weather was particularly favorable in June of 1922, and somewhat less so in June, 1919, for the development of the greatest amount of infection on barberry.¹⁰ The weather conditions of the other years were not quite so favorable.

The records of Mr. G. C. Mayoue, State Leader of barberry eradication in North Dakota, show that in 1919 there were found some 43 infected barberry bushes near and around Fargo. Altogether 162 barberry bushes were removed in Cass County that year. In 1920, 20 barberry bushes were discovered in the county and removed. In 1921 nine bushes were destroyed; a resurvey in that year revealed 139 sprouts, a goodly number of which were infected. In 1922, 93 bushes were found in the original survey, and 717 sprouts in a resurvey; most of these were badly rusted. All were destroyed. Still, in 1923 again 86 bushes were found and finally destroyed.

¹⁰ From unpublished data of Mr. L. W. Melander's thesis for the degree of Master of Science, submitted to the University of Minnesota in 1924.

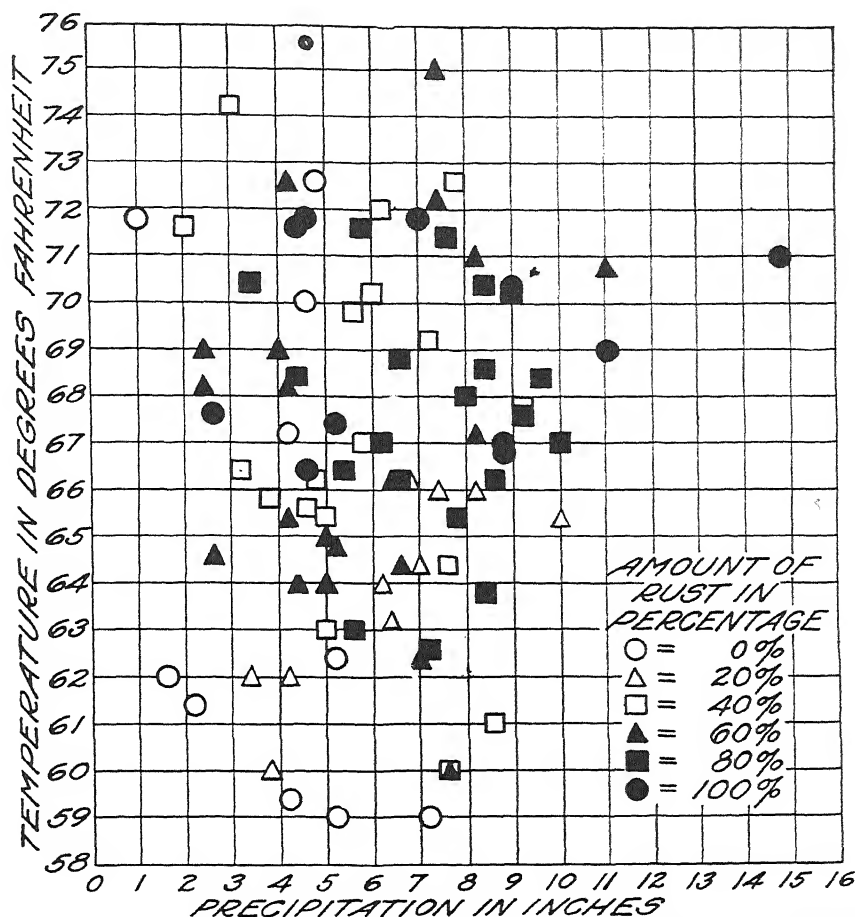


FIG. 37. Combined influence of mean temperature and total precipitation in last two months of wheat development on severity of stem rust infection, during the five years, 1919-1923, inclusive.

According to Christensen,¹¹ a bush of average size, about 6 to 7 feet high, has about 35,000 leaves. From actual counts approximately 80 per cent of the leaves were infected with rust, making a total of about 28,000 infected leaves on an average-sized barberry. Eight hundred leaves were selected at random, and on them there were 6,720 aecial clusters, averaging

¹¹ From unpublished results obtained by Dr. J. J. Christensen, Assistant Plant Pathologist, Agricultural Experiment Station, University of Minnesota, and Agent, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, in 1922, on barberries in Rice County, Minnesota.

8 + clusters for each infected leaf. As many as 222 aecial clusters were found on a single leaf. Counts on 350 clusters showed that there was an average of 36 cups per cluster, the actual number varying from 1 to 290. The number of spores in a single cup ranged from 8,000 to 15,000. Consequently, on the average infected barberry leaf there were produced 2,304,000 aeciospores, and on the average-sized bush 64,512,000,000 spores were produced. The last figure is about 40 times the population of the world.

It is not inconceivable then that the particularly heavy stem rust attack which prevailed at Fargo, N. Dak., from 1919 to 1923, inclusive, and the lack there of a consistent correlation between the severity of infection and the existing weather conditions, were due chiefly to the persistent presence in that vicinity of infected barberry bushes. The weather conditions did favor the development of the rust on the barberry, and an immeasurable quantity of aecial material was produced each year, so that there was really little chance for the susceptible varieties to escape heavy infection, unless the climatic conditions were totally unfavorable for the development of the rust on the grain, which was not the case.

Stability of Physiologic Forms under Diverse Cultural Conditions

There can be very little doubt but that the varieties of *P. graminis* are relatively fixed in their parasitism. It is evident from the exhaustive studies of Stakman and his coworkers (35, 34, 36, 27) and others, that these varieties do not change readily, if at all, genetically; that they do not seem easily to lose their inherent parasitic capabilities or gain increased virulence as a result of biotic, climatic, chemical, or physical influences. But do the physiologic forms of *tritici* variety behave in similar manner?

Uniformity of Behavior Over Long Periods of Time

A summary of the results regarding the action of several forms of *P. graminis tritici* cultured on the various differential varieties of *Triticum spp.* under a wide range of environmental conditions and over a long period of time is given in tables 57 and 58. Form 1 had been grown in the greenhouse as a stock culture for over eight years, covering nearly 150 urediniospore generations. A monosporous culture of this form was started in February, 1924, and since then has been grown in a spore-proof cage for 10 generations. Form 9 was grown as a multisporous culture in the greenhouse for approximately five years (over 100 urediniospore generations), and as a single-spore culture since March 1, 1924, (10 generations) in a spore-proof cage. Forms 29 and 38 have been cultured as stock strains only for about two, and one and one-half years, respectively, or for some 50-odd generations each.

TABLE 57.—Constancy of behavior of multisporeous cultures of physiologic forms of *Puccinia graminis tritici* grown in the greenhouse for many consecutive uredinospore generations

Experimental conditions	Reaction of differential varieties											
	Little Club C. I. 4066	Marquis C. I. 3641	Kanred C. I. 5146	Kota C. I. 5878	Arnautka C. I. 1493	Alindum C. I. 5296	Arnautka C. I. 6236	Rubanka C. I. 2094	Alme C. I. 5284	Blunkorn C. I. 2433	Vernal C. I. 3686	Rhapli C. I. 4013
<i>P. graminis tritici</i> form 1												
(Material collected Sept. 12, 1915; form identified Nov. 11, 1915; experiment concluded May 30, 1924)												
Number of trials	72	34	45	15	21	21	12	18	8	11	10	4
Plants inoculated	1127	336	439	121	186	181	109	154	74	93	86	30
Plants infected	1044	314	42	116	115	142	93	140	74	88	76	23
Minimum infection	3+	3	0	3-	0;	0.	0;	3±	3-	3	0.	0;
Maximum infection	4++	4++	0;	4-	1	1+	1+	4	4-	3+	1+	1++
<i>P. graminis tritici</i> form 9												
(Material collected July 27, 1918; form identified Feb. 22, 1919; experiment concluded May 30, 1924)												
Number of trials	63	22	30	18	19	25	13	30	11	10	32	11
Plants inoculated	880	188	257	177	161	199	116	276	110	100	353	89
Plants infected	771	145	24	155	151	170	111	218	109	99	295	74
Minimum infection	3	3±	0	3-	3±	3±	3±	3±	3-	3	3±	0.
Maximum infection	4+	4+	0;	4+	4+	4+	4+	4+	4±	4±	4+	1

TABLE 57.—(Continued)

Experimental conditions	Reaction of differential varieties											
	Little Club C. I. 4066	Marquis C. I. 3641	Kanred C. I. 5146	Kota C. I. 5878	Armutka C. I. 1493	Mindum C. I. 5296	Armutka C. I. 6236	Kubanka C. I. 2094	Acme C. I. 5234	Binkorn C. I. 2433	Vernal C. I. 3686	Rhapli C. I. 4013
<i>P. graminis tritici</i> form 29												
(Material collected June 23, 1922; form identified Oct. 20, 1922; experiment concluded Nov. 3, 1924)												
Number of trials	23	5	7	4	16	23	16	18	10	4	4	3
Plants inoculated	214	44	76	40	160	210	141	184	88	48	50	30
Plants infected	173	37	6	34	153	182	122	162	74	45	33	29
Minimum infection	4-	3+	0	3	0;	0;	0;	X=	X	3+	0	0;
Maximum infection	4++	4+	0.	4	4	4	4+	X++	4	4+	0;	1=
<i>P. graminis tritici</i> form 38												
(Material collected Aug. 10, 1922; form identified Mar. 8, 1923; experiment concluded Nov. 3, 1924)												
Number of trials	26	27	14	17	11	16	12	12	6	5	3	3
Plants inoculated	308	256	130	161	104	137	135	91	69	54	33	33
Plants infected	261	232	103	127	99	128	127	85	67	49	31	31
Minimum infection	4=	0;	3+	3=	0;	0;	X	X=	X+	3	0;	0;
Maximum infection	4++	2++	4++	3++	4+	4-	4+	4+	3++	4=	1-	1

" = infected areas frequently chlorotic, apparently on account of unfavorable climatic conditions;
" = normal uredinia often accompanied by a necrotic effect; infection somewhat similar to, but not identical with, "X" type; phenomenon evidently of genetic nature.

TABLE 58.—Correlation between the behavior of monosporous cultures of physiologic forms of *Puccinia graminis tritici*, grown in spore-proof cages, and that of multisporeous (stock) cultures of the same forms, grown in open greenhouse compartments

Type of culture	Experimental conditions	Reaction of differential varieties											
		Little Club C. I. 4066	Marquis C. I. 3641	Kanred C. I. 5146	Kota C. I. 5878	Armutka C. I. 1493	Mindum C. I. 5296	Armutka C. I. 6236	Kubanka C. I. 2094	Aene C. I. 5284	Elkorn C. I. 2433	Vernal C. I. 3686	Thaditi C. I. 4013

<i>P. graminis tritici</i> form 1 (Experiment commenced March 12, 1924; concluded Nov. 21, 1924)													
MONOSPOROUS	Number of trials	12	3	4	2	2	2	2	4	2	3	3	3
	Plants inoculated . . .	169	37	55	22	27	34	18	28	23	39	45	48
	Plants infected	153	37	6	21	21	33	17	25	23	35	35	43
	Minimum infection . .	4	4-	0	4-	0;	0;	0;	3	4=	4-	0;	0;
	Maximum infection . .	4++	4+	0.	4+	0;	0;	0;	4+	4++	4-	1+	1=
STOCK	Minimum infection . .	3+	3	0	3-	0;	0.	0;	3=	3-	3-	0.	0;
	Maximum infection . .	4++	4++	0;	4-	1	1+	1+	4	4-	3+	1+	1++

<i>P. graminis tritici</i> form 9 (Experiment commenced March 8, 1924; concluded Nov. 21, 1924)													
MONOSPOROUS	Number of trials	10	2	2	2	3	6	6	4	4	2	4	2
	Plants inoculated . . .	129	25	21	25	34	63	65	35	40	28	53	30
	Plants infected	125	18	1	25	33	59	65	33	40	24	53	17
	Minimum infection . .	4-	4-	0	3+ ^s	4=	3=	3+	3=	3=	3+	3++	0;
	Maximum infection . .	4+	4+	0	4	4+	4+	4+	4+	4	4-	4++	1-
STOCK	Minimum infection . .	3	3=	0	3=	3=	3=	3=	3=	3-	3	3=	0.
	Maximum infection . .	4+	4+	0;	4+	4+	4+	4+	4+	4=	4	4+	1

It will be seen from table 57 that there is a certain fluctuation in the type and degree of infection of each physiologic form on every one of the differential hosts. This fluctuation, however, is maintained within certain bounds regardless of the variation in environmental and cultural conditions. Stakman and Levine (30) have found that when a given host plant is resistant to a given form the infection range may extend from 0 to 2 ++; when a variety is susceptible, the infection may be from 3 = to 4 ++; when the reaction of a differential host is indeterminate, the infection is heterogeneous in nature and may fluctuate from x = to x ++ or even from 0 to 4 ++, depending on the prevailing conditions. As a result of subsequent work, it has been found that it probably would be better to designate those varieties on which the reaction ranges from 3 = to 3 + as "moderately resistant" rather than as "susceptible." While there is no hypersensitivity on such varieties, still the rust develops so poorly that the plant for practical purposes is at least moderately resistant. These authors found that all of the 37 forms of *P. graminis tritici* they described behave in like manner. Peltier's (23) intensive studies with two of the forms supplied him by the Minnesota laboratory fully substantiated the above results.

Monosporous cultures produced infection types quite similar to those produced by the stock cultures from which they were isolated, always falling fairly within the established range limits (See table 58). Multisporous cultures of form 9 often produce an infection on the durum varieties which approximates the "x" type. The monosporous cultures of this form act in like manner notwithstanding the additional protection against accidental contamination by means of "solitary confinement" in specially constructed spore-proof cages.

Consistency in Behavior of Different Strains of Identical Forms

Even though there may be some differences in the morphology of the urediniospores of individual strains of an identical physiologic form of *P. graminis tritici*, there is no very apparent dissimilarity in their action on the differential varieties of wheat. Forms 17 and 21 were selected to illustrate this point because of their wide distribution and frequent occurrence. Tables 59 and 60 show that there is some fluctuation in the action of the several strains of a given form on the various differential hosts but always within the range of infection of a single form when tested for many urediniospore generations under varying environmental conditions. Repeated cross inoculations from congenial to resistant differential hosts and *vice versa* did not affect in any way the pathogenicity of the physiologic forms in question.

It would appear, therefore, that the extensive and intensive investigations of this subject warrant the conclusion at the present time that para-

sitically and morphologically the different physiologic forms of *P. graminis tritici* are but little and only temporarily influenced by external conditions. Consequently it does not seem as though these forms could originate through a process of adaptation. As has been suggested by Stakman, Levine, and Leach (33), they very likely come into existence either through mutation or by hybridization.

There is as yet no definite experimental evidence that the physiologic forms of the stem rust pathogene arise as mutations. Further careful investigations along this line are now in progress. In 1918, Stakman Piemeisel, and Levine (36, p. 228) reported that upon inoculating *Berberis vulgaris* L. with teliospores from *Agrostis stolonifera* L. they obtained successful and normal infection on wheat, oats, and rye. The only physiologic form known to parasitize normally *Agrostis stolonifera* is *P. graminis agrostis*, whereas, wheat, oats, and rye can be attacked properly only by *P. graminis tritici*, *P. graminis avenae*, and *P. graminis secalis*, respectively. The percentage of infected plants was rather low, but this is generally the case when inoculations are made with aeciospores. Three different rust varieties then developed on the barberry from the one sown. The authors do not attempt to explain the results. They do suggest accidental contamination, although all possible precautions had been taken to prevent this. It is not impossible, therefore, that we are dealing here with a genuine case of segregation and recombination of genetic characters of a heterozygous rust in the aecial stage during the process of nuclear division and fusion. The general question of the origin of physiologic forms of *P. graminis tritici*, and their genetic nature in particular, are now being studied intensively.

GENERAL DISCUSSION

It may be seen from the results presented in this paper that almost all of the common wheat varieties grown in the uniform rust nurseries during the five-year period, 1919-1923, inclusive, were highly susceptible to black stem rust in the field. The durum varieties, as a class, were on the average much more resistant; the emmers were practically immune; Little Club was completely susceptible throughout. At least traces of stem rust were observed in all nurseries except those at Edmonton and Lacombe, Alta., in 1921. At Dickinson, N. Dak., in 1919, and at Akron, Colo., Scott, Sask., and Vermilion, Alta., in 1921, the maximum rust infection on any variety was recorded as a trace. But the fact that percentage estimates were obtained in 89 of the 95 experiments, or nursery years, is ample evidence of the prevalence of stem rust and the necessity for rust investigations in the wheat-growing regions.

Some of the physiologic forms isolated from the different nurseries were extremely virulent, while others were rather innocuous. In general, the

TABLE 59.—Agreement in behavior of separate cultures of *Puccinia graminis tritici*, form 17, collected in different years at different places

Cultures			Reaction of differential hosts													
Year collected	Place of collection	Acc. no.	Little Club C. I. 4066	Marguis C. I. 3641	Kanred C. I. 5146	Kota C. I. 5318	Arnakta C. I. 1493	Mindum C. I. 5296	Arnakta C. I. 6236	Kubanka C. I. 2094	Acme C. I. 5284	Elmkorn C. I. 2433	Vernal C. I. 3686	Rhapli C. I. 4013		
1919	Akron, Colo.	251	4	4+	0;	3	3	3	3	4	3	3	0;	0		
do	Mandan, N. Dak.	202	4	4	0	4	4	4	4	3+	3+	3	0;	0		
1920	Indian Head, Sask.	513	4	4	0	3	4	4	4	4	3+	4	1	1		
do	Mandan, N. Dak.	467	4	3	0	3	3	3	4	4	3+	4	1	1		
do	Rosthern, Sask.	532	4	4	0	3	4	4	4	3	4	4	1	1		
do	Saskatoon, Sask.	518	4	4	0	3	4	4	4	3	4	4	1	1		
do	Winnipeg, Man.	512	4	4	0	3	3	4	4	3	3	3	1	1		
1921	Brookings, S. Dak.	589	4	4	0;	4	4	4	4	4	3	3	0;	1		
do	St. Paul, Minn.	592	4	4	0	3	4	4	4	4	3	3	0;	1		
do	Edmonton, Alta.	650	4	4	0	3	4	4	4	4	3	3	0;	1		
do	Winnipeg, Man.	613	4	4	0	3	4	4	4	4	3	3	0;	1		
1922	Ames, Iowa	767	4	4	0	4	3+	4	4	4	3	3	0.	1		
do	Coon Creek, Minn.	773	4	4	0	3+	3+	4	4	4	4	4	0;	1		
do	Duluth, Minn.	807	4	4	0	3+	3+	4	4	4	4	4	0;	1		
do	Fargo, N. Dak.	789	4	4	0	3+	4	4	4	4	3+	3	0;	1		
do	Morris, Minn.	784	4	4	0	3+	4	4	4	3+	3+	3	0;	1		
do	Redfield, S. Dak.	777	3+	4	0	3+	4	4	4	3+	3+	3	0;	1		
1923	Mandan, N. Dak.	930	4	4	0	3	4	4	4	4	3	3	0;	1		
Range of infection for—																
Eighteen collections of f. 17																
Minimum infection			3+	3	0	3-	3	3	3-	3-	3-	3	0.	0		
Maximum infection			4+	4+	0;	4	4+	4+	4	4+	4	4	1	1+		
Prototype culture of f. 17																
Minimum infection			3	3	0	3-	4	3	3	3	3	3	0.	0.		
Maximum infection			4+	4+	0;	3+	4+	4+	4	4	4-	3+	1	0;		

reaction of the varieties in the greenhouse approximated their behavior in the field, whenever the same physiologic forms were concerned. No correlation between the virulence of the various physiologic forms on differential hosts and their prevalence and distribution could be definitely established. Some forms were widely distributed and of common occurrence: others were found infrequently and in restricted areas.

The distribution and prevalence of the different physiologic forms seem to depend on a multiplicity of factors. In the first place, it is quite obvious that resistant varieties of wheat, grown in a given region, undoubtedly impose a definite restraint on the occurrence of certain forms of *P. graminis tritici*. It also is very likely that the difference in climatic conditions in the various parts of the country plays an important part in the distribution of these forms. Finally, the common barberry may not be an insignificant factor, as long as there exists the possibility for stem rust forms to hybridize in the aecial stage.

There is convincing evidence that certain of the physiologic forms of *P. graminis tritici* differ not only parasitically but also morphologically; in anatomical structure as well as in infection capabilities. While the shape and size of spores of a given physiologic form evidently are due partly to true genetic factors, environment, as would be expected, affects their expression profoundly. For this reason physiologic forms can be distinguished best by their action on differential hosts.

The mean temperature and total precipitation of the last two months of the growing season can be definitely correlated with the intensity of stem rust infection on susceptible wheat varieties. However, the multiple correlation between these two weather conditions and the severity of the rust epidemic was only ± 0.4239 , from which it might be deduced that temperature and rainfall, although very important factors, are by no means the sole determiners of a successful stem rust epidemic.

The uneven quantitative distribution of the rust inoculum, the degree of maturity and general condition of the wheat plant at the time infection first takes place, the type of soil and method of fertilization, the right amount of dew fall at the critical moment, prevailing winds and sufficient sunshine, and finally the proximity of infected barberry bushes, all contribute to the extent and intenseness of the rust attack even on innately susceptible sorts.

External conditions affect but slightly and only temporarily either the pathogenicity or the morphology of physiologic forms of *P. graminis tritici*. It is improbable, therefore, that these forms originate through a progressive adaptation to changing environment. It is more likely that they come into existence as a result of occasional mutation or regular hybridization.

From the foregoing discussion it follows that the losses due to the stem rust scourge could be effectively reduced by the use of desirable resistant varieties, by the eradication of the alternate host, by judicious cultural practices, and perchance by the application of certain chemical dusts or sprays.

SUMMARY AND CONCLUSIONS

1. For the purpose of studying the effect of stem rust on different varieties of wheat under field conditions, and in order to determine the occurrence of physiologic forms of *Puccinia graminis tritici* in the spring wheat region, a series of uniform rust nurseries was established and operated at various experiment stations in the United States and Western Canada.

2. The maximum number of varieties of wheat and emmer tested in any one year was 24. The greatest number of stations at which uniform rust nurseries were grown in a single year also was 24. Altogether, during the five years, 1919-1923, inclusive, there were uniform rust nurseries at 34 different experiment stations.

3. The percentage of rust infection was estimated, whenever possible, just before the plants ripened, on the basis of the scale designed for this purpose in the Office of Cereal Crops and Diseases of the United States Department of Agriculture. Rust estimates in percentages were recorded in 89 of the 95 nursery years during which the experiment was in operation.

4. There was a distinct difference in the quantity of rust on different varieties, at different stations, in different years. In general, Acme, Monad, Pentad, and Kota seemed to withstand the stem rust attack in the field; Khapli always was highly resistant, and Vernal practically always so. The other varieties were susceptible in varying degrees.

5. In general, the reaction of the varieties in a given field nursery was the same as it was in the greenhouse to pure cultures of the physiologic forms isolated from the nursery in question. This, however, was not always true of Acme, Monad, Pentad, Kota, and Vernal, which often escaped infection under field conditions.

6. Nearly all of the hard red spring wheat varieties grown were susceptible to the prevailing physiologic forms. Of the durums, some varieties were considerably more susceptible than others, but as a class the durum varieties had a lower percentage of infection than either Marquis or the whole group of common wheats.

7. Rust specimens were collected each year from the different uniform nurseries and their identity was determined by the methods described by Stakman and Levine. Altogether 78 collections were studied; 20 of these consisted of two forms each, and one yielded three forms.

8. Nineteen different physiologic forms of *P. graminis tritici* were isolated from the collections made at the various uniform rust nurseries during the first five years of the experiment.

9. Little Club was completely susceptible to all of the forms isolated, in the greenhouse as well as in the field.

10. All of the forms isolated, except two, attacked all of the hard red spring varieties tested under greenhouse conditions. Marquis was resistant to one of the two excepted forms, and Kota was resistant to the other.

11. In the greenhouse, the durumms did not always behave as a group. Some of them were resistant to certain physiologic forms and susceptible to others, and *vice versa*. As a rule, the different durum varieties behaved differently. However, to some forms they were resistant *en masse*, and to others they were all susceptible.

12. Two of the physiologic forms isolated infected Vernal emmer normally under greenhouse conditions. None of the forms could infect Khapli emmer to any appreciable extent under any circumstance.

13. Einkorn was moderately susceptible to most of the forms isolated, but extremely resistant to two of them, *viz.*, forms 21 and 34.

14. Some of the physiologic forms isolated occurred frequently and were widely distributed; others occurred rarely and in limited areas. Physiologic form 17 was isolated from the greatest number of stations, and was to a greater or less extent a factor in the rust epidemics of the spring wheat region in each year between 1919 and 1923, inclusive. Form 21 appeared in almost as many different places as form 17, but was found in the uniform rust nurseries only during the last three years of the present experiment. Forms 5, 22, 34, and a few others, were procured only once each.

15. The frequency of occurrence of the different physiologic forms was not always coextensive with their distribution. In general, neither prevalence nor distribution of a given physiologic form could be directly correlated with the degree of its virulence on the differential varieties of wheat.

16. The prevalence of different forms of *P. graminis tritici* and the extent of their distribution are either inhibited or enhanced, by the climatic conditions prevailing in various parts of the country, by the wheat varieties grown in the different regions, and by the manner in which these forms originate and develop.

17. Eight physiologic forms of the *tritici* variety were chosen for a comparative morphological study. One hundred spores were found to constitute a representative random sample. The size and shape of individual urediniospores were determined and grouped in convenient classes. Monosporous as well as multisporous (stock) cultures were studied.

18. There appeared to be pronounced and significant differences in the size and shape of the urediniospores of some of the forms even when grown under identical conditions. These differences in many instances were considerably greater than the fluctuations induced in an individual form by altering the cultural conditions. They were practically always greater than the differences caused by a diversity in the geographic origin of two strains of a particular form.

19. No definite parallelism could be found between the differences in pathogenicity and the differences in morphology of the physiologic forms studied.

20. Even though there is a real morphologic basis for the distinction of physiologic forms of *P. graminis tritici* when developed under uniform conditions, they are most adequately identified by their parasitic behavior on certain varieties of wheat.

21. The difference between the size and shape of the urediniospores of the different physiologic forms of the *tritici* variety were sometimes as great as the differences in the size and shape of these spores of the composite forms (varieties) of *Puccinia graminis*; that is, different physiologic forms of *P. graminis tritici* often differ from each other morphologically quite as much as the *tritici* variety *per se* differs from the *avenae* or *secalis* varieties within the species *P. graminis*.

22. As the entities in the stem rust fungus now designated as varieties are specialized largely to different genera of Gramineae, whereas the physiologic forms of the varieties *tritici*, *avenae*, and *secalis* are differentiated by hosts within the genera *Triticum*, *Avena*, and *Secale* respectively, there seems to be sufficient warrant for regarding the former entities as of higher taxonomic rank than the physiologic forms. There is further justification for this procedure in the case of *P. graminis tritici*: (a) because there exists a close agreement between the average of the means of the urediniospore dimensions of the eight physiologic forms of *P. graminis tritici*, thus far biometrically studied, and the means previously determined for the variety as a whole; and (b) because of the negative correlation obtaining between the long and the short diameters of the urediniospores of all the forms within the *tritici* group, regardless of cultural and environmental conditions—in other words, the morphogenic processes being characteristic for the entire group.

23. Other conditions being equal, the uneven quantitative distribution of the rust inoculum is a factor in the difference in the severity of infection on naturally susceptible varieties. Furthermore, the severity of a stem rust epidemic seems to depend on the degree of maturity and general con-

dition of the wheat plant at the time infection first takes place; it also depends indirectly on the type of soil, its topography, and fertilization.

24. The extent and severity of stem rust epidemics depend also on favorable weather conditions, such as suitable atmospheric temperature, plenty of precipitation and soil moisture, the right amount of dew fall at the critical moment, prevailing winds, and sufficient sunshine.

25. There is evidently a positive correlation between the mean temperature of the last two months of the growing season and the total rainfall during this period and the severity of stem rust infection on susceptible varieties. When the total precipitation fell below 2 inches, or the temperature was less than 60° F., no rust developed. Not more than 40 per cent of stem rust, as a rule, appeared at or below 62° F. Over 90 per cent of rust was observed only where the mean temperature ranged between 66° F. and 72° F. and the total rainfall exceeded 2.5 inches.

26. Inasmuch as the multiple correlation coefficient for the combined effect of temperature and rainfall during the last two months of the development of wheat on the intensity of stem rust epidemics exceeds only slightly + 0.4, indicating a reduction in variability of less than 10 per cent, it is apparent that the totality of the other factors, essential for a successful epidemic, is of major importance. To determine mathematically the combined effect of all contributing factors, much more observation and experimentation is required.

27. The common barberry is a constant and persistent menace to the small grain crops of the spring wheat region, not only as a source of supply of immeasurable quantities of inoculum but possibly also as a breeder, and certainly as a propagator, of virulent physiologic forms of the stem rust parasite. Therefore *Berberis vulgaris* and its stem-rust-bearing allies should be eradicated with all possible haste and thoroughness.

28. The parasitism of the physiologic forms of *Puccinia graminis tritici* is but little and only temporarily affected by external conditions. That these forms originate through progressive adaptation to changing environment is not borne out by extensive experimentation. Intensive studies of their possible origin by means of mutation or through the processes of hybridization and anastomosis are now under way.

29. Resistant varieties apparently are effective and feasible means for overcoming the ravages of black stem rust. The choice of varieties will depend in each case upon their adaptability to local conditions and the market value they command, in addition to their resistance to stem rust and other diseases and pests.

30. In conjunction with the attempt to destroy the alternate host and the effort to secure desirable resistant varieties, serious consideration should

be given to judicious cultural practices, such as the use of early maturing sorts, adequate crop rotations, and proper fertilization methods, and to the possibilities of obtaining effective chemical remedies.

BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
IN COOPERATION WITH
AGRICULTURAL EXPERIMENT STATION,
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ST. PAUL, MINN.

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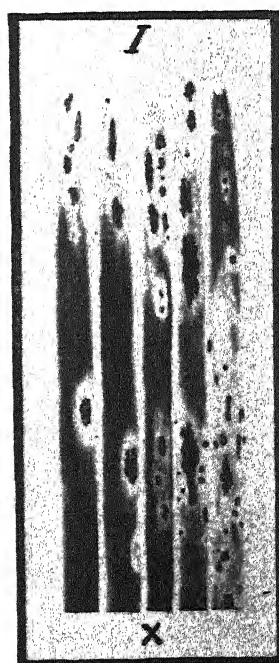
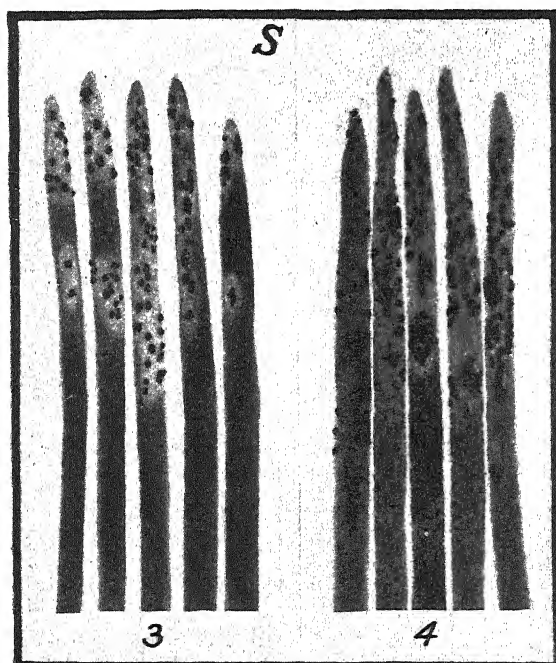
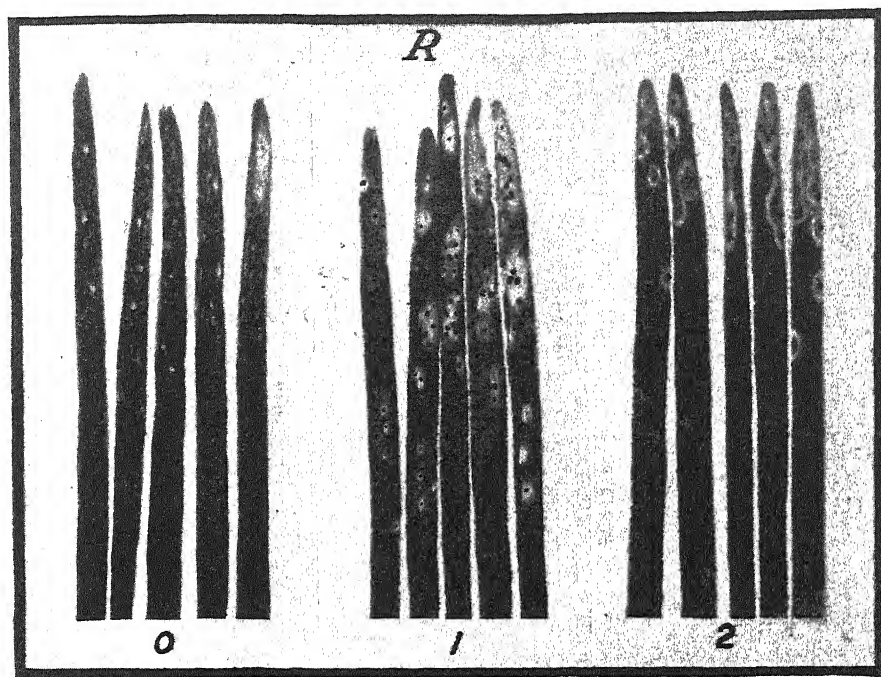
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EXPLANATION OF PLATE I

Classes of host reaction: Resistant (R), Susceptible (S), and Indeterminate (I), as indicated by different types of infection (0, 1, 2, 3, 4, and X), produced by physiologic forms of *Puccinia graminis tritici* on varieties of *Triticum spp.*

- 0.—Either no infection whatever, or pronounced hypersensitive flecks;
- 1.—Minute uredinia within solid necrotic lesions;
- 2.—Small pustules in green islands surrounded by necrotic halos;
- 3.—Medium sized uredinia, slight chlorosis but no necrosis;
- 4.—Very heavy infection, large confluent uredinia;
- X.—Infection heterogeneous and rather ill defined.



PHYTOPATHOLOGICAL NOTES

Emil Chr. Hansen-Medal, 1928.—Pursuant to the last will and testament of the late Professor Emil Chr. Hansen and his wife, a fund bearing his name has been established, the statutes of which were ratified by the Government on June 17, 1911.

At proper intervals, as a rule every two or three years, beginning in 1914, a Gold Medal bearing his effigy and accompanied by a sum of at least 2,000 Kroner is to be awarded on the donor's birthday, the 8th of May, to the author of a distinguished publication on some microbiological subject that has appeared in recent years in Denmark or elsewhere.

The fund is committed to the administration of the chiefs of the two departments of the Carlsberg Laboratory, together with a Danish biologist elected by the governing body of the Carlsberg Laboratory.

The person to whom the medal is to be assigned shall be designated by a committee composed of the above-mentioned trustees of the fund and at least two foreign microbiologists who, at the request of the said trustees, have declared themselves willing to act on the committee.

The medal was awarded in 1914 to Professor Dr. Jules Bordet, Brussels, for researches in medical microbiology; in 1922 to Professor Dr. M. W. Beijerinck, Delft, for researches on general microbiology; in 1923 to Dr. E. I. Allen, Plymouth, for researches in marine microbiology.

In 1928 it is proposed to award the medal to an author of bacteriological or mycological phytopathology.

Dr. O. Appel, Dahlem b. Berlin, and Professor Johanna Westerdijk, Utrecht, have been made members of the committee.

All the communications regarding the fund should be sent to the President of the Board of Trustees.

Board of Trustees:

Professor C. O. Jensen, M.D. and D.V.M., Government Veterinary Surgeon, Serum Institute of the Royal Veterinary and Agricultural College, Copenhagen.

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Professor S. P. L. Sørensen, Ph.D. and M.D., Chemical Department of the Carlsberg Laboratory, Copenhagen, President of the Board of Trustees—S. P. L. SØRENSEN, *Copenhagen-Valby, Denmark.*

First Report of the Occurrence of Black Scab or Warty Disease of Potato in Belgium.—Black scab or warty disease of potato was found in two places in Belgium at approximately the same time during the season of 1927: at Courcelles in the province of Hainaut and at Stavelot in the province of Liège.

In both cases the disease was found in the small holdings of laborers, where potatoes are grown year after year on the same plots for home use.

The competent services of the Ministry of Agriculture have immediately taken all measures required.—E. MARCHAL, *Gembloux, Belgium*.

ABSTRACTS OF PAPERS PRESENTED AT THE NINETEENTH
ANNUAL MEETING OF THE AMERICAN PHYTOPATHO-
LOGICAL SOCIETY, NASHVILLE, TENN., DE-
CEMBER 28, 1927, TO JANUARY 1, 1928

Overgrowths and hairy root on nursery apple and quince trees. J. H. MUNCIE and W. B. SHIPPY.

Isolation trials from 111 grafted apple trees with hairy knots at the union failed to yield *Pseudomonas tumefaciens*. In similar trials on 58 trees showing the fibrous form of hairy root in the absence of an overgrowth, the pathogen was recovered in four cases from the swollen bases of the hairy roots, and from one of 100 apple trees showing the burr-knot type of hairy root on the stock, but not from 20 quince trees and 15 rooted quince cuttings. Typical hairy roots were induced at the nodes of 15 quince cuttings by rooting under aseptic conditions.

Of 2,200 apple seedlings inoculated in the field 7 per cent produced galls. Isolations from representative specimens were positive.

Overgrowths with fleshy hairy roots developed on 0.4 per cent of two lots each of 2,400 wounded seedlings grown in two plots of field soil non-infested and infested with *Ps. tumefaciens*, but the pathogen was not recovered. Hairy roots in the absence of overgrowths developed equally on wounded and unwounded seedlings in infested and non-infested soil. Isolations from seedlings showing either hairy roots or woolly knots were negative.

Isolation studies of burr-knots were made from specimens of 24 varieties of apple from four states. Rarely were organisms found resembling *Ps. tumefaciens*. These were not pathogenic tomato plants. Field observations show that, while certain environmental conditions favor the production of burr-knots, certain varieties have never shown them. Certain varieties showing no burr-knots, when cross-pollinated, produced hybrids bearing aerial tumors at an early age.

Grafting as a further means of preventing callus knots on apple. I. E. MELHUS, J. H. MUNCIE, and VERNON C. FISK.

In a graft with an overhanging scion tip, excess callus first forms as a knot from the severed cambium of the scion, later growing backward, filling the space between stock and scion. The phellogen layer is soon differentiated in the callus. This cuts off and causes the death of the outer layer of proliferating cells of the scion callus. The slower growing stock callus finds no meristematic cells with which to unite, and lack of continuity in the union results. Further growth of the callus knot results from the laying down of phloem and xylem by the cambium layer differentiated in the callus. These facts coupled with field observations showing that approximately 60 per cent of the overgrowths at the union of piece-root grafted trees occurred at the tip of the scion lip led to trials of the wedge or cleft graft as a means of holding the scion lip in place and thus preventing knot formation.

Storage trials were made in which wedge and tongue grafts of the variety Wealthy were callused at low, medium, and high temperatures. The wedge grafts showed an average of 75 per cent less callus knots than the tongue grafts. In nursery field trials one-year-old, wedged-grafted trees of the variety Whitney had 4 per cent with callus

knots; while 28 per cent of those from tongue grafts were knotted. At the end of two years the remaining 362 wedge- and 348 tongue-grafted Whitney trees were dug. Of the wedge grafted trees, 8.1 per cent showed callus knots, and of the tongue grafted 32.8 per cent. In both cases the majority of the knots developed at the tip of the scion lip.

Studies of the history of development of wound overgrowths on apple grafts and of the influence of wrappers on their suppression. A. J. RIKER, W. M. BANFIELD, and G. W. KEITT.

Frequent observations of apple grafts throughout the first season showed that certain enlargement began to develop very early as the direct result of poor union. These are rather easily controlled by good fitting and wrapping. Enlargements were also observed to be initiated during or after midseason on any part of the union. The development of certain of these seems to have been correlated with periods of rapid secondary thickening.

The duration of strength of wrappers in the soil has been shown to affect the development of wound overgrowths. Studies were made of various wrappers, with the following results: (1) by chemical treatments cotton thread was made to endure for any desired period; (2) girdling ensued in Wisconsin from wrappers which lasted more than 11 weeks; (3) typical wound overgrowths followed girdling; (4) uneven application considerably lengthened the durability of wrappers; and (5) of the wrappers used, medical adhesive tape gave the highest percentage of salable trees. A series of experiments in Wisconsin, Minnesota, Nebraska, Missouri, and Oklahoma involving trials during three years has consistently shown that grafts wrapped with an adhesive tape produce a considerably higher percentage of salable trees than those wrapped with string.

Correlation of the wound overgrowth and crown gall of apple in parts of Europe and of the United States. A. J. RIKER.

A survey was made in certain nurseries of England, France, and Holland in order to correlate the wound overgrowth and crown gall situations in these regions with those in the North-central and Northeastern United States. While examining the results of this survey one must hold in mind such considerations as the following: (1) that this part of Europe has a comparatively mild climate with a long growing season; (2) that the market requires a very high percentage of dwarfed trees; and (3) that practically all fruit trees are propagated by budding. As in America, the practice of budding has practically eliminated wound overgrowths from the plants except as these developments follow injuries to the stock. Bacterial crown gall was found widely distributed but of no economic significance except in a few isolated cases. On the other hand the common practice of employing Paradise and Doucin stocks for the growth of more or less dwarfed apple trees made burr-knots very common. Likewise, enlargements at the union several inches above the soil line, which were attributed to lack of congeniality, were quite usually present on dwarfed stock.

Studies on the life history of the crown gall organism. W. M. BANFIELD.

Studies are being made of various phases of the life history of *Bacterium tumefaciens* in relation to raspberry crown gall.

The pathogen, as previously reported by other workers, has been found in very large numbers in tissue removed aseptically from the interior of crown galls. In many cases masses of the bacteria have been observed on the surface of galls. They have

been obtained in considerable numbers from water in which galls had been placed for a short time. Thus it appears that crown galls may be a continual source of inoculum to the surrounding medium.

The pathogen has been found to live in different types of unsterilized soil for periods varying from a few months to over a year, and to overwinter in soil in the field at Madison, Wisconsin. As in all cases mentioned in this abstract, the identity of the organism was established by isolation in pure culture and successful inoculation into tomato.

Experiments as to the mode of penetration of the pathogen into disease-free raspberry plants grown in infested soil have shown that wounds are important avenues of entrance, and have suggested that both chewing insects and growth characters of certain varieties of raspberry may play important rôles in infection.

The method and rate of migration of Bacterium tumefaciens in tomato. J. BEN HILL.

Vigorous healthy young stems of tomato were inoculated with *Bacterium tumefaciens*. Study of properly stained paraffin sections of this material secured within three hours after inoculation shows that the bacteria migrate as zoogloae or zoogloal strands through the inter-cellular spaces of the pith and subepidermal chlorophyllose parenchyma. The zoogloae apparently consist of semi-fluid gelatinous matrix densely packed with bacteria. The ends of the zoogloal strands are blunt and rounded. Zoogloal strands are continuous for from 0.35 to 0.56 mm. and continuous but for slight interruptions for much greater distances. Over 80 distinct zoogloae were measured and their rate of migration calculated. Rates of movement of the zoogloae of 0.04 mm. per minute for the first 15 minutes and of approximately 0.03 mm. per minute for three hours were calculated. The maximum rate of migration of the zoogloal strands, while comparatively rapid, is only one twenty-fifth of the published calculation of the rate of a single motile *B. tumefaciens* in liquid medium.

Longevity of Pseudomonas tumefaciens Sm. & Town. in various soils. M. K. PATEL.

In the laboratory *Pseudomonas tumefaciens* was reisolated after 16 months from sterilized clay, loam, and quartz sand, which were artificially infested. The number of colonies on agar poured plates of crystal violet bile decreased as the time increased. No difference in the number of colonies were found in plates from loam and clay.

Pure cultures of the pathogen were also obtained from non-sterilized infested sand after 16 months and from clay and loam after 14 months. This decrease in longevity may be due to the fact that the pathogen had to compete with a greater number of soil organisms in loam and clay than in sand. The unsterilized and sterilized samples of infested loam kept in the open from October to March yielded virulent cultures of the pathogen. The samples of unsterilized and sterilized infested clay loam and quartz sand, buried 12 inches in the soil from November to March, gave pathogenic cultures of the crown gall bacteria. Field soils of the loam type infested in late fall with a 72-hour broth culture of the pathogen and finely chopped tomato galls yielded the pathogen the following March.

Strains of Pseudomonas tumefaciens Sm. and Town. and their prevalence in various soils. M. K. PATEL.

Further studies were carried out on 15 non-pathogenic strains of *Pseudomonas tumefaciens*. Sm. and Town. These strains and a pathogenic one gave practically the same titre when tri- and tetra-valent salts were used in agglutination tests. Six of these

non-pathogenic strains and the one pathogenic strain had one, two, or three polar flagella. The pathogenic strains always, and the non-pathogenic ones never, produced crown galls on sweet peas, garden peas, oleander, bryophyllum, raspberries, weeping willows, and apples. One pathogenic strain which was identical in all other respects with the other pathogenic strains lost its virulence after being cultured two years on common medium.

Using the author's crystal violet bile medium, plates were poured from various soil samples. From 41 of the 96 samples collected in 12 nurseries in 9 states, organisms resembling *Pseudomonas tumefaciens* were obtained. Seven of these organisms proved pathogenic on tomato plants. Only one non-pathogenic colony which resembled *Pseudomonas tumefaciens* was obtained from another lot of 14 samples taken from pasture and corn land, virgin prairie, and nursery fields on which no crown gall susceptible crops had been grown for many years.

Repeated isolations from water and air gave no colonies resembling *Pseudomonas tumefaciens*.

Studies on crown gall transplants. MICHAEL LEVINE.

Pieces of crown gall tissue of *Ricinus communis*, when transplanted into the same and other *Ricinus* plants, produce small crown galls in most cases.

Ricinus crown gall tissues, when transplanted to growing portions of the tobacco plant, also produce galls. Similar results were obtained with crown galls of tomato, geranium, and beet. These neoplasms are generally of the host type. The crown gall on the tobacco, induced by an inoculum of tomato or *Ricinus* crown gall, becomes differentiated and forms leafy shoots with characteristic tobacco leaves.

Small, maroon-colored pieces of sugar beet crown gall transplanted to the yellow mangel produce a relatively large number of yellow pigmented crown galls. In a few cases only, there is indisputable evidence of the growth of the inoculum. There are also distinct proliferations of the host, as shown by gross sections of the tumor.

These studies show that the crown galls are not generally formed as the result of the growth of the transplanted tumor issue itself, but follow the introduction of *Bacterium tumefaciens* with the inoculum. Plate cultures of parts of the inocula used yielded *B. tumefaciens*, as determined by cultural studies, smears, and subsequent inoculations.

Rumex crispus, a weed host of *Pseudomonas tumefaciens*. J. H. MUNCIE.

In June, 1926, a plant of *Rumex crispus* L. with a large overgrowth at the crown was taken by Dr. O. H. Elmer from a field of raspberries in Minnesota, some plants of which were infested with crown gall. Isolations were made from the gall, and *Pseudomonas tumefaciens* was recovered in almost pure culture. Inoculations from these plates into healthy young tomato plants resulted in the production of typical crown galls. Further inoculations into *Rumex crispus* with strains of *Ps. tumefaciens* from *Rumex* and raspberry resulted in the formation of galls in all cases.

As far as the writer is aware, this is the first report of crown gall occurring in nature on a species of *Rumex*. This suggests the possibility of infected perennial weeds as a means of carrying over the crown gall in fields planted to non-susceptible crops.

The effects of lime on cigar tobacco. C. M. SLAGG, J. E. MONTEUIL, and T. G. MAJOR.

The effects of air-slaked lime at the rate of 2,000 pounds an acre in combination with manure; with commercial fertilizers; and with commercial fertilizers and manure

have been studied on cigar tobacco at the Farnham, Quebec, Experimental Station in 1925, 1926, and 1927. The field used had grown several crops of tobacco previously in a three-year rotation of oats, clover, and tobacco. The soil was free from *Thielavia* root rot at the beginning of the experiment. After three years of continuous tobacco culture a moderate infestation of *Thielavia* was present. It was more marked on the limed plots. Significant decreases in yield and quantity were secured wherever lime was applied during each of the three years of the experiment.

Soil reaction and black root rot of tobacco. P. J. ANDERSON.

Black root rot (*Thielavia basicola*) is present in all old tobacco fields of the Connecticut Valley, irrespective of reaction. Severe reduction in yield, however, occurs only in soils which approach the neutral point. Injury below pH 5.6 is practically negligible. Practically all old fields with a reaction about pH 6.0 are suffering from root rot; the higher the reaction, the smaller the growth. In the "doubtful zone" between pH 5.6 and pH 6.0 there may be no injury or it may be present in varying degrees, as influenced by other characters of the soil and the season. A rapid soil-testing technique has been developed and is being used extensively by the growers in choosing fields to be set to tobacco each year.

The influence of cropping systems and fertilizers on black and brown root rot of tobacco. J. P. JONES.

Different cropping systems have not influenced black root rot of tobacco; but have affected the brown root rot, the extent depending upon the other crop grown in the rotation. In the animal-husbandry rotation, tobacco, following either hay or corn, was so severely attacked by the brown root rot as to cause low yields. Tobacco grown in the money-crop rotation after onions or potatoes, or in continuous culture without a cover crop, was damaged but little by brown root rot. Timothy as a cover crop induced brown root rot and reduced the yield. Tobacco grown every year on the same land and without a cover crop showed the least injury by brown root rot and the best yields.

Black root rot has been found to do no damage on soils more acid than pH 5.95. When the soil was made less acid than this by liming, this disease became harmful. Phosphoric acid also acted as a stimulant to black root rot in a soil already badly infested, but it did not act in this manner on an acid soil. Carbonate of potash, to furnish the amount of potash required for tobacco, when applied to acid soils, has not influenced black rot. Little evidence has been obtained to show that brown root rot is affected by fertilizers.

Effect of timothy infusion of different ages on the growth of tobacco and on brown root rot of tobacco. W. L. DORAN.

When decoctions of timothy in water were applied to soil in which tobacco plants were growing, the effect on the plants was either stimulatory or inhibitory, depending on the age of the decoctions. Infusion of whole timothy plants, kept at about 27° C., aged 1 to 3 weeks, did not inhibit growth of tobacco and did not induce brown root rot. But these infusions, when 4 to 10 weeks old, produced on tobacco the symptoms of brown root rot and greatly inhibited growth. Dry weights of plants receiving timothy infusions 4 to 8 weeks old were 2 to 33 (relative numbers) as compared with 100 in checks. Infusions 9 to 10 weeks old were less toxic, dry weights of corresponding tobacco plants being 61 to 77 (relative numbers).

In another experiment, timothy decoctions were made with most of the tops removed and were kept at about 16° C. Under these conditions, infusions 1 to 6 weeks old did

not induce brown root rot and greatly improved the growth of the tobacco plants. But when these infusions were 7 to 12 weeks old their application resulted in symptoms of brown root rot. When infusions were 10 to 12 weeks old the growth of the plants was reduced fully 50 per cent.

Accuracy in comparing various concentrations of tobacco-mosaic virus. FRANCIS O. HOLMES.

An investigation of ultraviolet light photography applied to plant viruses emphasized the desirability of knowing whether the small amounts of fluid represented in the photographs could be expected to contain as much as, or more than, one effective unit or the active agent.

By carrying virus from expressed juice or diseased plants to small healthy plants by single inoculations with fine No. 00 black enamel insect pins, minute quantities of juice are transferred, and transmission is secured regularly enough to allow quantitative studies. Two factors are believed responsible for the uniformity of the readings, i. e., uniform wounding, and deposit in the wound of a large proportion of the transferred virus. Small test plants, 50 in a flat, were employed, thus insuring numerous determinations. To adjust the dosage, and facilitate inoculation, several insect pins may be mounted in one handle and used simultaneously.

The method is so accurate that test dilutions of 1:4 or 1:8 can be identified from undiluted samples of virus with a certainty corresponding to odds of 22 to 1 or more. Similarly adjacent portions of infected plant tissues can be identified and accurate readings made with no more than 1,000 seedlings. Equally accurate comparisons of sources differing even less in strength can be made by using larger numbers of plants.

Experiments and observations on the control of true tobacco mosaic. W. D. VALLEAU and E. M. JOHNSON.

In a rotation series in which 13,000 plants are set each year, mosaic counts, made about three weeks after setting, for three consecutive years, showed 9, 6, and 8.7 per cent mosaic, respectively, when plants were pulled by men chewing natural leaf tobacco. The next year, when the men chewed mostly commercial tobacco, the percentage was 2.1. The two following years, when sterilized tobacco was chewed, the percentages were 0.44 and 0.05 respectively. The third year the hands were washed before the pulling was begun. Last season (1927), using these precautions, 32 mosaic plants developed in 71,183 plants set. A survey study of the relationship between chewing habits of workers and percentage of mosaic gave the following results:

3.10 per cent mosaic in 100,993 plants pulled by uncleanly worker who chewed tobacco.

0.64 per cent mosaic in 12,515 plants pulled by cleanly worker who chewed tobacco.

0.55 per cent mosaic in 20,963 plants pulled by worker who did not chew tobacco.

Of 134 plants pulled by a man who chewed viruliferous natural-leaf tobacco and occasionally spit on his hands while pulling, mosaic developed on 108; while 156 plants pulled with clean hands were healthy 47 days after setting. When no effort was made to contaminate the worker's fingers, 878 plants developed 39 cases of mosaic. When 837 plants were pulled with clean hands and set in alternate rows, but one case of mosaic developed in 48 days.

Some virus diseases of tobacco in Kentucky. W. D. VALLEAU and E. M. JOHNSON.

Four apparently distinct strains of true tobacco mosaic have been obtained from tobacco in Kentucky. Mild and severe strains have been carried through two years and

have remained distinct. Recently a milder and a more severe form were found. Virus diseases of tobacco, distinct from mosaic, and common near Lexington, are: "ring-spot," which has been transferred from naturally-infected tobacco, a bull-nettle, and cucumbers to tobacco, but not from ring-spotted delphinium and peony; "Coarse-etched," a disease somewhat similar to ring-spot; "Puffed," which has been transferred from naturally infected tobacco, cucumbers, muskmelons, and milkweeds to tobacco (possibly cucumber mosaic); "Etched," characterized by chlorotic dots in the growing point and a fine etched necrotic pattern on some of the leaves; "Etched+," evidently a severe strain of etched; and "Vein-margin," which may be a very mild strain of etched. It causes slight spotting in the growing point and a general mild chlorosis of some of the older leaves, except in the tissue immediately adjoining the veins, thus leaving a dark band along the small veins. These diseases are not so prevalent as tobacco mosaic, but, where present, they appear to spread as rapidly by natural means and to be equally injurious.

Tobacco ringspot; a virus disease with a wide host range. S. A. WINGARD and F. D. FROMME.

Further studies of tobacco ringspot have shown it to be a virus disease capable of infecting a wide range of plants. Infection has been obtained on 19 genera of plants representing the following 11 families: *Solanaceae*, *Compositae*, *Leguminosae*, *Phytolaccaceae*, *Violaceae*, *Amaranthaceae*, *Chenopodiaceae*, *Polygonaceae*, *Convolvulaceae*, *Plantaginaceae*, *Aizoaceae*. There is some evidence that the green aphid may transmit the virus in the greenhouse, but insects are not necessary for infection, for it is readily accomplished when trial plants are swabbed with the expressed sap of diseased plants. The sap has been found infectious in dilutions as high as 1 in 10,000 but not in higher dilutions.

Cucumber fruit-rot and angular leaf-spot. GEORGE F. WEBER.

During the past five years, observations have been made and data collected in Florida on the occurrence and association of these two diseases of cucumbers, caused by *Bacterium lachrymans* E. F. S. and Bry., which have appeared in the state each year to a greater or less extent. The data show that angular leaf spot in the field may or may not be accompanied by fruit rot, and that fruit rot is always accompanied by angular leaf-spot. In fields planted with seed disinfected for 10 minutes with 1:1000 corrosive sublimate the disease did not develop on leaf or fruit, but the disease appeared in 75 per cent of fields planted with untreated seed.

Two bacterial organisms isolated from diseased cucumber leaves and fruits collected near Gainesville, Florida, were used to inoculate cucumber leaves and fruit under controlled conditions. The organism isolated from leaves produced both the angular spot on leaves and the characteristic fruit-rot; and the organism isolated from fruit produced both the fruit rot and the angular leaf-spot. The two organisms show as much similarity in pure culture as two transfers of the same culture. These studies indicate that a single organism causes both the leaf spot and fruit rot of cucumbers as discovered by O. F. Burger.

Preliminary report on a new leaf spot of pecan. O. C. BOYD.

In 1926, in several pecan orchards near Albany, Georgia, a characteristic leaf spot was observed that had not previously been reported. It was observed in 1927 in practically every pecan section surveyed in the southern part of the state.

It was more common in orchards than in nurseries. The following varieties, listed in the order of their susceptibility, have been found affected: Delmas, Money-maker, Stuart, Frotcher, Van Deman, Schley, Alley, Mobile. Common varieties observed not attacked are Moore, Success, Tesch, and Pabst.

The trees are injured by premature, partial defoliation. The spots when young are pale yellow in color, later turning to yellowish brown, or perhaps brown to black, depending on the variety concerned. Conidia are produced in from one to many minute ascervuli on the underside of the lesion, forming under certain conditions a mildew-like or downy coating over the spot.

The causal organism, while resembling to some extent both a *Cylindrosporium* and a *Cercospora*, does not seem typical of either of those genera. Demaree and Cole consider the fungus identical with *Cylindrosporium caryigenum* Ell. & Ev., described as occurring on *Carya amara*, and collected in Canada in 1899. (Plant Disease Reporter, Vol. 11, No. 11, 1927.

Cotton seed treatment by the dusting method. NAOMI CHAPMAN WOODROOF.

At the Georgia Experiment Station treating undelinted cotton seed with fungicidal dusts has been found to be economical and much simpler than the standard method of delinting with sulphuric acid and soaking for 30 minutes in mercuric chloride. Of the numerous dusts tested several have been found capable of surface-sterilizing the seed. Mercuric resinate and a combination mercuric chloride dust are equally as effective as the standard treatment. Angular leaf spot was very much reduced by seed disinfectants. Yields have not been materially increased by any of the treatments. Further work is necessary before definite recommendations can be made.

Outstanding wilt-resistant cotton varieties. D. C. NEAL.

Wilt-resistance tests were made in 1926 and 1927 with 18 varieties and selections of staple and short cottons on soil which had been artificially inoculated so as to insure more complete exposure of the plant roots to the wilt fungus, *Fusarium vasinfectum*. Since these experiments have been in progress, considerable difficulty has been experienced in finding resistant varieties which yield well under varying conditions. Sometimes a variety is very resistant to wilt, but may not be poliflorous, or is late maturing, or unsuitable for areas where the boll weevil is a serious factor.

A list of both staple and short cotton varieties which have shown considerable resistance in the tests during 1926 and 1927 and which have produced well on wilt-infested soil is given below with the source of the seed.

WILT-RESISTANT STAPLE COTTONS

Super-Seven	Coker Pedigreed Seed Company, Hartsville, South Carolina.
Lightning Express	Humphrey-Coker, Hartsville, S. C.
D. & P. L. No. 6	Delta & Pine Land Planting Company, Scott, Mississippi.
Watson's Long Staple.....	L. O. Watson, Florence, S. C.

WILT-RESISTANT SHORT COTTONS

Cook, Rhyne	Rhyne Bros., Benton, Alabama.
Dixie-Triumph	L. O. Watson, Florence, S. C.
Miller	Miss. Experiment Station, A. & M. College, Miss.
Cleveland 54	Miss. Experiment Station, A. & M. College, Miss.
Triumph, Willis	Hinds County Farm Bureau, Jackson, Miss.

The use of resistant varieties and liberal fertilization of the crop with balanced fertilizers are considered to be the most feasible and economical means of controlling the disease.

The gummosis of sugar cane. MELVILLE T. COOK.

Gummosis of sugar cane is a vascular disease caused by *Bacterium vascularum* E. F. S. Some varieties of cane are very susceptible while others appear to be immune. Some varieties are killed out by the organism and in others the yield is very much reduced. It is said that the cell walls are dissolved, but our studies indicate that this is rare and occurs only in young tissues. The tracheary tubes are filled with the organism, which is frequently abundant also in the parenchyma. In the mills, gummosis causes a lowering a purities and interferes with crystallization. The leaf symptoms are unreliable, as they do not always appear on diseased cane and similar symptoms due to other causes are sometimes found on healthy cane. Field experiments show that the disease spreads readily from row to row. On plantations it is probably carried mechanically. No insect carriers have been discovered in Porto Rico.

Sugar cane eye spot in Cuba. JAMES A. FARIS.

Two distinct leaf spots of sugar cane due to different species of *Helminthosporium* occur in Cuba. Eye spot, present in Western Cuba since 1915, appears to have been re-introduced recently into Eastern Cuba from Porto Rico. It injures susceptible varieties seriously, producing reddish spots surrounded by a lighter halo and progressing to the edge of the leaf by long, reddish, broad bands. The cane varieties D 109, F. C. 137, F. C. 214, and F. C. 306 are too susceptible for commercial growing. More resistant are Badila, Cristalina, Java 105, Java Unknown, and Uba. Environmental conditions greatly influence the development of eye spot, cane in localities with high humidity, as in low, foggy valleys, being worst affected. This common eye spot, a major disease of sugar cane, has been reported from Hawaii, Fiji, Australia, Formosa, The Philippines, Java, India, Mauritius, Cuba, and Porto Rico. No evidence of it was seen during recent visits to Louisiana and Florida, and it probably does not yet occur in the Southern United States.

Brown stripe of sugar cane in Cuba. JAMES A. FARIS.

Brown stripe of sugar cane, caused by a species of *Helminthosporium*, different from that causing eye spot, attacks Cristalina cane throughout Cuba, appearing as minute, reddish spots which elongate to form characteristic linear stripes surrounded by slight halos. This previously has been considered an immature stage of the eye spot described in the preceding abstract. That the two diseases are not the same has been demonstrated by inoculating many plants both under bell-jars and grown in glass cylinders under sterile conditions. The two types of spots can be distinguished in their early stages. The causal fungi also have distinct characteristics in growth on various media and in spore morphology. A similar brown stripe was observed in Louisiana and Florida the summer of 1927, and the causal fungus may be the same as that named *H. stenospilum* by Drechsler. Slow growth seems to favor the development of brown stripe, its prevalence being most marked during long dry periods in the summer and latter part of the winter.

A species of Helminthosporium distinct from Helminthosporium sacchari, causing brown stripe of sugar cane. CHARLES DRECHSLER.

Diseased sugar cane specimens collected over several years in Georgia, Florida, and Cuba reveal two species of *Helminthosporium* causing foliar injury. Lesions in some Cuban material appearing as elliptical reddish-brown spots, in well developed instances often 20 mm. long and 3 mm. wide, with somewhat bleached central and zonal markings, yield under moist conditions a fungus producing fuliginous conidia with thin peripheral

wall and 3 to 10 septa (average 6.7), measuring 32 to 103 μ in length (average 71 μ) by 9 to 17 μ in width (average 14 μ). Under similar conditions lesions in leaves from Georgia, Florida, and Cuba, appearing first as very narrow, linear brown streaks, later often becoming more extensive through enlargement or coalescence without conspicuous internal markings, yield a form producing dark olivaceous conidia with thick peripheral wall and 3 to 12 septa (average 7.7), measuring 40 to 128 μ in length (average 83 μ) by 12 to 22 μ in width (average 17 μ). The first-mentioned fungus from lesions of true "eye-spot" type is the one more aptly identified as *H. sacchari* Butler; the second, associated with "brown stripe," and distinguished by its much darker, thicker-walled, broader spores, is provisionally named *H. stenospilum* n. sp.

The effect of disinfectants upon the germination of seeds kept in storage for indefinite periods after treatment. C. R. ORTON.

The seed of several varieties of wheat, oats, sweet corn, field corn, and beans which were treated with chemical disinfectants in liquid and dust form at various dates from November, 1924, to November, 1926, and stored at 20–22° C. were germinated October, 1927, in greenhouse soil and two sets of comparisons made—(1) with the germination of untreated seed of the same source, (2) with the germination of the same lots of treated and untreated seed at the approximate date of treatment.

The organic mercury dusts did not decrease germination and often increased it after periods of one to three years. Copper carbonate dusts were injurious on Navy and Black Valentine Beans. Dusts composed of $HgCl_2$ and oxide of copper caused decreased germination in some cases. Liquid treatments with organic and inorganic mercuries and water are more likely to be injurious, water being especially so. Formaldehyde was especially injurious to oats.

A bacterial disease of broad beans. A. J. RIKER.

Studies were made in England of a bacterial disease of broad beans. An organism was isolated consistently and in large numbers from lesions commonly called "chocolate spots." Two strains of this organism were run through the cycle of isolation, inoculation, infection, and reisolation five successive times each. Typical symptoms developed under certain conditions following inoculations in the greenhouse. When all conditions were favorable, such as temperature between 20° and 30° C., relative humidity approaching saturation, plants vigorously growing, and cultures actively motile, plants were killed in three days. The cultures were also vigorously pathogenic to garden peas.

The organism grows rapidly on ordinary media. Nutrient agar colonies after five days are circular, radiately ridged, umbonate, undulate, and granular. When the medium contains considerable water and dextrose, the colonies tend to become rhizoid. According to the 1924 chart of the Society of American Bacteriologists this organism has the numbers 5011, 32100, 0202. A non-pathogenic organism having the same number was found in association.

Histological studies showed that the organism multiplied rapidly inside the tissue and occupied primarily an intercellular position. After inoculation the bacteria sometimes spread rapidly in the vascular elements.

Irrigation as a cause of white spot of alfalfa. B. L. RICHARDS.

White spot frequently becomes a serious factor in alfalfa production in Utah. The nature and cause of this disease has remained obscure and, although various theories as to its etiology have been advanced, no experimental data have been presented in support

of any of them. Recent studies indicate clearly that the trouble is in some way associated with an unbalanced water relationship in the plant which may readily be induced, under certain conditions, by the application of irrigation water. During 1926 and 1927 white spot was experimentally produced over a considerable acreage and to an extent of 75-80 per cent of the plants in certain fields. These experiments were so controlled and were repeated in such numbers as to justify the conclusion that, while other factors might be responsible under local conditions for the production of white spot, the improper application of irrigation water is by far the most important cause of the disease in the West. Surveys conducted preceding and subsequent to the experimental work confirm this conclusion and indicate that heavy rains may also be responsible at times for this disease.

The production of similar, if not identical, lesions in the leaves of sweet clover (*Melilotus alba*) in the same field and under the same experimental conditions responsible for the white spot of alfalfa indicated that such localized mesophyll destruction is not peculiar to alfalfa.

Mercury as a control for turf diseases. JOHN MONTEITH, JR., and A. S. DAHL

Experimental work on control of large brown-patch (*Rhizoctonia solani*) and small brown-patch (*Rhizoctonia* sp.) of turf was continued at the Turf Garden, Arlington Farm, Virginia. The results substantiated those previously reported; namely, a large number of both organic and inorganic mercury compounds are, with the exception of the sulphide, effective against these diseases, their effectiveness depending chiefly on the mercury content. Preliminary tests with metallic mercury, mixed with chalk, indicated that mercury in this form gave as good results as when applied in a chemical compound. Observations again failed to disclose any injury to turf due to an accumulation of mercury in the soil similar to that found with copper. On the other hand, applications of mercury to a soil may prevent an attack of disease for weeks or even months. A heavy application of calomel August 31, 1926, prevented small brown-patch until the following August. Corrosive sublimate put on in October, 1926, at Madison, Wisconsin, likewise prevented attacks of snow mold during February and March, 1927. Calomel was again found to give longest protection per unit of mercury, to be least likely to burn, but slower in checking an active case of brown-patch. Mercury compounds may be combined with fertilizers used in ordinary green maintenance without impairing the fungicidal or fertilizing properties.

Leafhopper injury of legumes. JOHN MONTEITH, JR.

Experiments in insect-proof cages at Arlington Farm, Virginia, demonstrated that potato leafhopper (*Empoasca fabae*) injury occurs on most of the leguminous forage crops, as well as on common beans. Symptoms identical with those observed in the field developed in hopper-infested cages; whereas plants in hopper-free cages remained vigorous and healthy. In all infested plants there is a dwarfing of the vegetative parts and a reduction in floral development. Tip and marginal burning of leaves is a common symptom among the legumes. Some plants, as *Melilotus*, suffer comparatively little discoloration of the leaves; while others, such as clovers, show pronounced yellowing or reddening, especially at the tips and margins. Beans, cowpeas, and soybeans, in addition to the yellowing and burning symptoms, show a crinkling and curling of the younger leaves resembling that described on beans inoculated with curly-top virus. Entire plants are frequently killed, but usually the injury is localized. Even badly injured plants may recover entirely. Although probably not of the virus class, this damage is apparently caused by something more than the mere mechanical injuries due to penetration and

sucking. Striking differences in varietal susceptibility were observed in all the crops under observation. Resistance appears to be due to some quality in the host plant which makes it less favorable for the feeding and reproduction of the leafhoppers.

The effective methods of eradicating Rhamnus species susceptible to Puccinia coronata
Corda. S. M. DIFTZ and L. D. LEACH.

Rhamnus cathartica and *R. lanceolata* have initiated local epidemics of crown rust in the Upper Mississippi Valley during 11 of the past 12 years. Table 1 shows four effective methods of eradicating *R. lanceolata*, i. e., application of salt to base of plant; application of kerosene to base of plant; application of salt following removal of top growth; and removal of the crown. One hundred and thirty-five individual bushes were treated by the first, 105 by the second, 25 by the third, and 67 by the fourth method.

TABLE 1.—*Effective methods of eradicating Rhamnus lanceolata.*

Area of cross-section of main stems in sq. in.	Bush standing		Top growth removed	Crown removed
	Pounds of salt	Quarts of kerosene		
1-2	2½	½		
2-5	5	1	Chopped off and 2½	No additional treatment
5-15	10	2	pounds salt added	
15-35	15	3	Chopped off and 5	
35-55	20	4	pounds salt added	
55-125	25	5		

The response of *Rhamnus cathartica* to salt and removal of the crown was similar to that of *R. lanceolata*.

Seasonal effect on rate of killing *Rhamnus lanceolata* was shown by the fact that bushes treated with salt in March died on an average within 66 days; those treated in July, within 98 days; those in August within 162 days. It required an average of 110 days to kill the bushes treated with kerosene in March, 77 for those treated in July, and 109 for those in August. (Cooperative investigations by the Iowa Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.)

Inheritance of resistance to Puccinia sorghi in maize. E. B. MAINS.

Selections of Golden Glow 228, Golden Bantam 996, and Howling Mob 983 are resistant to physiologic form 1, of *Puccinia sorghi* and susceptible to physiologic form 3 in the seedling stage, while selections of Golden Glow 208 are resistant to both forms. The inheritance of the resistance of the selections has been studied in a number of crosses with varieties susceptible to both physiologic forms of the rust. The ratios obtained in the F₂, for the most part, closely approximate 3 resistant; 1 susceptible indicating a single factor pair in each case. A study of the inheritance of resistance in relation to endosperm color and texture, albinism, and anther ear dwarf has so far given no evidence of linkage with these characters. The same factor is apparently responsible for the resistance of selections of Golden Glow 208 to both physiologic forms 1 and 3. (Cooperative Investigations between the Purdue Agricultural Experiment Station and the Office of Cereal Crops and Diseases, United States Department of Agriculture.)

New physiologic forms of Tilletia tritici in wheat. E. F. GAINES.

In 1927 bunt appeared on hitherto immune strains of wheat at five different stations in Washington, Oregon, and Montana.

An experiment planned to test the comparative pathogenicity of *T. tritici* from Germany with that common to Eastern Washington showed unmistakable differences. The American wheats were much more susceptible to the German form, while the German wheats succumbed more readily to the American form.

Inoculation tests in Europe indicate that the so-called immune wheats of America are sometimes infected.

The gradual increase of bunt in America, especially in Kansas, Virginia, and Pennsylvania, during recent years may be due to new forms which are more virulent on the wheats commonly grown there. (Cooperative investigations by the Washington Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.)

New dust treatments for oat smuts. J. D. SAYRE and R. C. THOMAS.

Two new dust treatments for the control of the smuts of oats were tried last summer with excellent results. One of the dusts was made by mixing formaldehyde with infusorial earth in different concentrations. This dust, when used at the rate of three ounces a bushel of grain, gave just as good control of the smuts of oats as did the wet-formaldehyde method. The formaldehyde-dust plots showed less than 0.2 per cent smut, while the checks averaged 47 per cent smut. The other dust was made by mixing finely ground solid iodine with infusorial earth. The iodine is quickly vaporized at ordinary temperatures and diffuses through the infusorial earth and is absorbed by it. When used at the same rate as the formaldehyde dust, only three smutted heads of oats were found in the three one-hundredth-acre plots. No injury to the grain resulted from either treatment. Further tests on the minimum concentration of the dusts necessary to control smuts of wheat and oats are under way. The cost of treating grain with these dusts is estimated at less than five cents a bushel.

Seed disinfectants for the control of covered smut and stripe of hulless barley. R. H. PORTER.

Extensive experiments were carried on at Nanking, China, in 1925 and 1926 with seed disinfectants for the control of covered smut and stripe of hulless barley. Liquid treatments used were Tillantin "B," and Uspulun 0.3 per cent solutions, cold formaldehyde, and hot formaldehyde. Dry treatments were copper carbonate, Tillantin "B," Uspulun, and powdered copper sulfate. In 1925, all treatments reduced the covered smut to less than 0.5 per cent, in rod rows. The checks had 7 per cent smut. In rod-square plots the checks had 27 per cent of smut and those treated with copper carbonate (54 per cent) had 1.2 per cent. In 1926 copper carbonate and dry Uspulun eliminated smut entirely; whereas dry Tillantin "B" gave 0.87 per cent. The checks averaged 6 per cent. The percentage increase in yield due to copper carbonate, Uspulun, and Tillantin "B" were 15.4 per cent, 12.4 per cent and 20.7 per cent respectively in 1926. Average yield of the checks was 13.0 bushels per acre. Plantings were in rod rows with 9 replications. Copper carbonate, Uspulun dust, and Tillantin dust, when used on stripe infected seed, reduced the stripe from 10.8 per cent in the checks to 5.5, 4.0, and 4.0 per cent respectively. Yield increases were 10, 9.6 and 10 bushels per acre, respectively, in plots of $\frac{1}{2}$ square rod with 3 replications. Average yield of checks was 45.3 bushels an acre.

A preliminary method of measuring the relative efficiency of seed corn disinfectants.
W. P. RALEIGH.

In studying seed-corn disinfectants it is often necessary to conduct many preliminary tests in order to ascertain the desirable concentrations and to eliminate undesirable dusts before making field trials. The following method has been found of much value. Treatments are made on units of 15 kernels each, placed in crystallizing dishes (10×5 cm.), and covered with 4 centimeters of washed sand. An equal amount of water is added to each culture at the beginning and later if necessary. The cultures may be placed either in the laboratory (22° to 25° C.) or in the greenhouse (16° to 20° C.). Both nearly-disease-free and diseased seed are used in these seed-treatment tests, the former to determine injury and the latter to determine the effectiveness of the fungicides. The advantages of this method are: (1) ease of application at any time of year, and without the use of greenhouse or field space; (2) visibility of both roots and tops so that the effect of disease or injury by the disinfectant can be correlated with top growth at any rate; (3) ease of freeing the plants from the sand for final examination; (4) decrease in number of fungicides to be used in further experiments.

Transmission of potato spindle-tuber by grasshoppers (Locustidae). R. W. GOSS.

While aphids have been shown to transmit spindle-tuber they are not numerous enough in the potato sections of western Nebraska to account for the rapidity with which the disease sometimes spreads. Tests were therefore made with some of the more common insects occurring in potato fields. In 1925, out of 36 inoculations made using grasshoppers, from 1 to 10 in each test, as the transmitting agents, 8 resulted in typical spindle-tuber. In 1926, 29 infections were obtained from 64 inoculations. Single, double, and triple inoculations with the same grasshoppers also were tried. With larger numbers of insects and repeated inoculations the percentage of infection increased. Uncaged control plants always remained healthy and the possibility of uncontrolled field transmission by other means is negligible. Current season symptoms did not occur in many cases and the progeny were indexed in the greenhouse and planted in the field the following year to obtain these results. All insects were fed upon healthy plants before being used in the tests. In the 1926 experiments it was found that 5 of the 77 plants upon which grasshoppers were fed before being placed on spindle-tuber plants became infected. Apparently these insects were viruliferous when collected.

A new and destructive disease of the potato in Utah and its relation to the potato psylla.
B. L. RICHARDS.

During the past season a new and destructive disease swept Utah potato fields, the west slope of Colorado, and later appeared in southern Idaho, Montana, and Wyoming. The rate of spread and the degree of damage indicate that the malady may become one of the most destructive diseases so far reported on the potato in this region. The early crop was most severely affected. Symptoms of the disease clearly differentiate it from any of the potato diseases thus far described. An upward rolling of the basal portion of young leaves constitute the first and most distinctive symptom. In the Bliss Triumph and Irish Cobbler this rolled portion becomes brilliantly colored, varying from light pinkish yellow to purple. The older leaves roll upward, turn yellow, and die. The axillary buds are stimulated into one or a combination of three types of growth; thick shoots which may exceed the leaf in length, aerial tubers, and rosettes of small and frequently highly colored leaves.

In Utah, Idaho, Montana, and Wyoming the potato Psylla (*Paratrioza cockerelli* Sulc.) was found associated with diseased plants, and subsequent experimental work

shows the disease to be induced in some way by the nymph of this insect, all the various symptoms of the disease noted in the field having been produced under controlled conditions in the greenhouse. Nymphs confined by gauze bags to a single lower or older leaf produce the early symptoms of the disease in remote portions of the plant in 9 days.

Because of the peculiar yellowness common to the disease in all varieties of potato so far studied, the writer suggests the name "yellows."

A preliminary report on the relationship of insect carriers to the development of tip and margin burn of Irish potatoes. H. R. ROSEN.

Results of three years of experimental work at Fayetteville, Arkansas, with relatively large numbers of Irish potato plants placed under wire screens indicate a market correlation between vegetative growth and the use of such screens. Plants underneath the screens appear much larger, darker green, and are longer lived than unscreened plants. Quantitative studies of the chlorophyll content indicate that greater amounts of green coloring matter are present in the screened plants. When unscreened plants show relatively large proportions of tip- and margin-burning of the leaves, screened plants show little. Are these differences due to the effect of shade, of wind-breakage, or of insect control? Preliminary work appears to indicate that insect control, particularly of leaf hoppers, is at least partly concerned in these differences. This is suggested by recent studies, in which plants that were not completely covered with screens, and hence open to attack by leaf hoppers, showed the same amount of tip- and margin-burnning as plants free from screens. Also, plants partially covered with wooden slats showed no superiority over unshaded ones.

The properties and behavior of potato rugose mosaic. JAMES JOHNSON.

With a view towards more satisfactory descriptions and classification of potato virus diseases, the properties of certain of these viruses are being determined. The rugose mosaic virus for instance will not yield good infection at a dilution much greater than 1 part of mosaic plant extract to 10 parts of water. The extracted virus loses its infectivity rapidly *in vitro*, approximately half of it being lost after 6 hours and all after 24 hours. The thermal death point of the rugose mosaic virus lies close to 43° C.; hence it is extremely sensitive to heat, as it is also to chemicals. Varieties of potatoes differ in their susceptibility to the virus. Bliss Triumph and Green Mountain being the most susceptible and Rural New Yorker the most resistant of the varieties tried. All attempts to pass this virus through bacterial proof filters have thus far failed. Other possible diagnostic features are also being studied. In general, the properties of the potato rugose mosaic virus are quite different from those of certain other potato viruses studied, such as leaf-rolling mosaic and spot necrosis.

The reaction and treatment of soils infested with Plasmodiophora brassicae Wor. F. L. WELLMAN.

It has been commonly believed that clubroot of crucifers is primarily dependent on the presence of an acid soil reaction, which if neutralized tends to reduce the amount of the disease. An extensive survey of seriously diseased fields shows reactions ranging from pH 5.0 to 7.7, in which most cases are close to neutral or slightly alkaline. Laboratory and field studies have demonstrated that increase of pH is not alone effective in inhibiting this disease. The amounts of different neutralizing substances required to inhibit the disease seem to depend upon the choice of reagents rather than the effect on pH. The pH was varied experimentally to alkalinity well above the high point found

in naturally infective soils without reducing severity of attack. Treatment of soils with different types of lime showed that 2 tons of calcium hydrate to the acre produced practical control of clubroot on a commercial scale. Air-slacked lime was found usable, but not satisfactory; while raw limestones, marls, and gypsums were ineffective.

A fusarium-resistant cabbage of Jersey Wakefield type. J. C. WALKER and F. L. WELLMAN.

There is a growing need in certain yellow-infested areas for an early-maturing resistant cabbage variety of the pointed head type. Initial selections were made in 1925 from surviving heads of the Jersey Wakefield variety on infested soil. These were grown to seed in the greenhouse during the following winter. Of the resulting progenies, tested in 1926, all segregated in the approximate ratio of 3 resistant to 1 susceptible. All poor type strains were discarded, and selections made from progenies of 4 original plants were grown to seed in 1926-27. In 1927, from 12 selfed progenies tested, 6 were secured which were homozygous for resistance, all showing a complete stand of healthy plants. The remaining 6 progenies again segregated at approximately 3 resistant to 1 susceptible. The results confirm earlier evidence that *Fusarium*-resistance in cabbage is a single dominant Mendelian character. From the homozygous resistant families of 1927 several were selected for multiplication. They are typical of Jersey Wakefield in all important characters including earliness.

The use of selection and selfing in improving Iacope cabbage. I. E. MELHUS and D. R. PORTER.

A strain of the yellows-resistant cabbage Iacope has been developed by selection and self pollination which proved immune when grown on severely infested land in 1927. This strain, designated as 15-23-2-S1-S1-S1, was subjected to a severe test for resistance to yellows for three growing seasons, and in 1927 every plant of 159 resisted the disease. The relative resistance of this strain since the original selection of Iacope from Copenhagen Market is as follows:

Generation	Percentage resistant	Index of resistance
1	33	206
2	45	145
3	85	304
4	95	452
5	100	455
6	100	323

This strain was compared with 12 other strains of Iacope and with Golden Acre and Copenhagen Market in 1927. Each strain was replicated four times on severely infested soil, and while this strain appeared immune, Golden Acre showed only 33 per cent resistance and Copenhagen Market only 32 per cent. This strain also possesses other desirable characteristics of a good early cabbage. It is the earliest of the Iacope strains, produces the largest percentage of marketable heads, and produces heads of medium size, uniform as to size and shape, and of uniform leaf characteristics and color.

Aphis transmission of cucumber mosaic. S. P. DOOLITTLE and M. N. WALKER.

Experiments on the transmission of cucumber mosaic by the melon aphid, *Aphis gossypii*, indicate that virus-free aphids can transmit the disease after feeding for five minutes on a mosaic plant. When transferred to healthy plants these aphids produced infection after an equally brief period of feeding. Experiments also indicate that the aphid loses its ability to transmit mosaic immediately after it first feeds on a healthy plant. In the latter experiments 1 to 10 aphids reared on mosaic plants were fed for 10 to 20 minutes on a healthy plant and immediately transferred to another healthy plant, where they remained. Infection occurred regularly on the first plants, but the second plants remained healthy in nearly every case. The aphids on the latter plants lived and multiplied, thus eliminating the possibility that the absence of infection was due to injury to the aphids. It appears that aphid transmission of mosaic results from the virus being carried into the plant tissues on the insect's proboscis and that the minute amount of virus thus carried is exhausted during the first feeding period. Aphids from mosaic plants do not transmit the disease after 6 to 8 hours' confinement in a test tube, this period being approximately the same as that during which the expressed juices of mosaic cucumber plants remain infectious.

Further evidence of resistance to cucumber mosaic in the Chinese cucumber. R. H. PORTER.

In July, 1927, 200 cucumber plants of the variety "Chinese Long" and 50 of the American Variety White Spine were inoculated in the greenhouse with the virus of cucumber mosaic. All plants were transplanted to the field two weeks after inoculation and were observed throughout the summer. None of the Chinese plants developed symptoms of mosaic but 75 per cent of the White Spine did. In this same field three rows of White Spine plants were grown from seed planted in hills. In these the incidence of mosaic due to natural infection was about 50 per cent, so that there was ample opportunity for natural as well as artificial infection to take place on the transplants of Chinese cucumbers. Furthermore, two and one-half rows were planted with the seed of the Chinese variety and in no case did any symptoms of mosaic develop. All the plants in this field were dusted while young with calcium arsenate and lime to restrict the injury from beetles but no effect was made to control plant lice.

Studies with the watermelon wilt, caused by *Fusarium nivum* E. F. S. D. R. PORTER.

Fusarium nivum E. F. S. may enter the host through root hairs, wounds, and epidermis of the hypocotyl. It produces 4 well defined symptoms on seedlings: (1) seedling rot, manifested by failure of the seedlings to emerge after germination; (2) damping-off, manifested by necrosis of cortical tissues at the soil surface causing the seedling to fall over; (3) stunting; (4) wilting, yellowing of the cotyledons, and eventual death. The organism grows most rapidly on potato dextrose agar between 24° and 32° C., its minimum being above 8° C. and its maximum above 35° C. It grows rapidly on a wide range of acid and alkaline media. The rate of growth on media of different pH values expressed in terms of the diameter of the colonies after 6 days is as follows:

pH 3.0	trace	pH 5.4	8.00 cm.
3.3	1.1 cm.	5.8	7.63
3.8	3.84	6.8	7.10
4.2	5.66	8.4	5.8
4.6	7.23		

The organism will remain alive (no visible growth) in an oxygen free chamber for at least 20 days. This fungus has been isolated from watermelon seeds secured from 12 sources and directly from seeds taken from melons attached to wilted vines. Cultures of *Fusarium nivium* secured from 5 states seem to be equally pathogenic to watermelon seedlings though differing slightly in physiological reactions in culture.

Infection in the field occurs most rapidly when seeds are planted about $\frac{1}{2}$ inch above the inoculum. Inoculum placed above the seeds produces relatively little early infection.

Varietal resistance of watermelons to wilt (Fusarium nivium E. F. S.). D. R. PORTER.

During 1926 and 1927, 52 varieties of watermelons, 9 strains of African forage melons, the preserving citron, and 13 strains of Chinese melons were tested for wilt resistance on severely infested land in Iowa. All commercial varieties tested proved very susceptible. Infection varied from 75 to 100 per cent. The Conqueror was the most resistant. It lived about three weeks longer than the other varieties. Susceptibility among these varieties seems to vary with the soil and with moisture and temperature conditions.

Four of the 13 Chinese selections secured by R. H. Porter at Nanking, China, appeared to possess some resistance in 1927. Their resistance is expressed by the percentages 25, 28, 29, and 39 respectively; while commercial varieties used as controls showed 100 per cent infection. The preserving citron proved to be 82 per cent resistant in 1927 and two selections from African forage melons appeared to be immune. First generation hybrids seem to be as susceptible as the susceptible parent. Resistance in the F_2 generation varies with the resistant parent. African hybrids are much more resistant in the F_2 generation than are Conqueror hybrids. Flesh quality of the resistant parent appears to be dominant in the F_1 generation.

Some cytological phenomena in disease-resistant plants. J. DUFRENOY.

Certain species of *Phytophthora* live primarily in meristematic tissues in the cambial regions of roots and stems. As soon as the invading tips of the hyphae enter any part of the cambial tissue, they induce cells at some distance away to divide in a transverse plane and to form a pathologic cambial layer. This layer consists of two types of cells: (a) those on the side away from the infected tissues, containing large nuclei, dense cytoplasm and mitochondria; (b) those on the side toward the infected tissues, soon developing large vacuoles which accumulate tannoid compounds, fuse and crowd the cytoplasm and nuclei to the peripheries of the cells. If the fungus grows rapidly enough to invade the meristematic cells of the pathologic layer, it will continue to thrive, deriving its food from the cells and at the expense of their vital activity. If, on the contrary, the fungus fails to penetrate the pathologic layer before the cells have developed large vacuoles containing tannic compounds, it cannot obtain food and therefore starves. In this manner the lesion is checked.

Bacteriophage of Bacterium pruni. H. W. ANDERSON.

A bacteriophage of high potency was obtained from soil beneath infected peach trees. Ten successive filtrations were necessary to secure a bacteriophage sufficient to clear turbid cultures with certainty. A stock supply capable of clearing turbid suspensions of *B. pruni* when diluted to 10^{-6} and in some cases to 10^{-10} was finally obtained. Typical plaques were obtained with high dilutions on agar plates but secondary cultures almost always appeared after several days. One effort to get a bacteriophage from old infected peach leaves was unsuccessful.

The cycle of infection in apple blotch. EDWIN J. KOHL.

In an experimental block of 60 young Duchess apple trees at Vincennes, Indiana, in which cankers have been excised since 1922 and blotch sprays have been withheld from 1925 to 1927, counts of petiole infection were made in August, 1927, and the origin of infection determined. It was found that on 37 trees which showed infection and on which 12,330 leaves were examined, no infection was traced to cankers on 1926 wood; infection on 1615 leaves was traced to 72 cankers on 1925 wood; no infection was traced to 2 cankers on 1925 wood; infection on 35 leaves was traced to 6 cankers on 1924 wood; no infection was traced to cankers on 1923 or 1921 wood, of which two each were found present. This indicates the cycle of infection is at least two years in length.

By the use of potted trees it was found that infection at LaFayette, Ind., occurred during 18 out of 27 rain periods between 3 days and 7 weeks after petal-fall (May 7). At Mitchell, Ind., in 1927 infection occurred during 15 out of 17 rain periods between 5 days and 6 weeks after petal-fall (April 25).

Sporotrichum fruit spot of apple. MAX W. GARDNER.

In Grimes, Ben Davis, and Winesap apples grown in southern Indiana, a shallow surface spotting, apparently of fungous origin, has been found when the fruit was removed from cold storage. The lesions are more or less circular, slightly sunken, light brown spots, 5 to 15 mm. in diameter, with an indistinct margin and a silvery area at the center. The fungus which has been found constantly associated with these lesions closely resembles *Sporotrichum malorum* Kidd and Beaumont, a culture of which was obtained for comparison. An abundance of mycelium and spores is produced within the rotted tissues and the fungus can be readily isolated in pure culture. It grows rather slowly, but well at low temperatures. Inoculation tests have shown that the mycelium will invade the uninjured fruit and produce small lesions around the lenticels.

Studies of black root rot of apple. F. D. FROMME.

The occurrence of black root rot of apple in Virginia and other parts of the United States is discussed. The causative agent, *Xylaria mali* nom. nov., is an active parasite producing disease and death of trees of normal vigor. Death of 28 per cent of trees of the initial stand has occurred in one orchard at 25 years of age, and replant deaths have equalled 42 per cent at seven years of age. Stromata of the fungus are formed during late stages of disease. The fungus is also actively parasitic on Norway maple and Mahaleb cherry. It produces more limited infection on certain other deciduous trees. Some progress has been made in the attempts to control this disease through development of resistant root-stocks.

Factors important in the development of perithecia of Venturia inaequalis. E. E. WILSON.

The studies previously reported (РНУТОРАТН. 16: 77) have been continued during the past two years. The data obtained further emphasize the relationship of the time of leaf-fall to the time of maturity of ascospores the following spring. In both years ascospores matured earlier in leaves which were placed on the ground in September than in those similarly placed later in the autumn. The delay in maturation did not, however, correspond to the delay in leaf-fall, as a delay in leaf-fall was followed by a shortening in the time between leaf-fall and maturation of ascospores.

Temperatures and moisture are cardinal factors in development of perithecia. The optimal temperature for their initiation was near 13° C., while the optimum for maturation

tion of ascospores was near 20° C. Growth of asci was found to occur more readily at 4° and 7° C. than maturation of ascospores. Development of the perithecia was sharply checked when the moisture of the leaf fell below an undefined limit. Perithecia matured more rapidly and normally in leaves which were alternately wet and dry than in those which were continuously wet.

Perithecia matured somewhat earlier in leaves of certain varieties than of others, although all varieties under observation produced mature perithecia in nature.

The type and abundance of leaf lesions appeared to bear a direct relationship to the quantity of perithecia produced. No evidence was found that perithecia were produced at points remote from lesions or that the fungus spread to uninfected leaves and there produced perithecia.

Fall applications of fungicides in relation to apple scab control. G. W. KEITT and E. WILSON.

The studies previously reported (PHYTOPATH. 17: 45) have been continued and extended. Severely infected apple foliage was sprayed after harvest but before leaf-fall with various materials, including calcium arsenite, Paris green, calcium silicofluoride, sodium silicofluoride, and certain proprietary preparations of chlorophenol mercury of different solubilities. Lime, Kayso, bordeaux mixture, and other materials were added to these chemicals in various combinations to modify their properties. Representative samples of untreated and treated leaves were overwintered and noted for the occurrence of perithecia of *Penturia inaequalis*. Numerous perithecia developed on untreated leaves. Comparatively little inhibition of their development seemed to result from applications of the silicofluorides or chlorophenol mercury. Marked reduction in perithecial development followed the use of calcium arsenite and Paris green, respectively, each in various combinations with other materials. In certain cases the treated leaves developed less than 10 per cent as many perithecia as the untreated. Calcium arsenite, unless modified by adding appropriate materials, caused considerable host injury. The results do not warrant specific recommendations for post-harvest fungicidal applications for apple scab control but they seem to give much promise of progress through attacking the fungus at a hitherto neglected, potentially vulnerable stage in its life-history.

Certain sulphur fungicides in the control of apple scab. J. M. HAMILTON and G. W. KEITT.

The following fungicides were tested for controlling apple leaf infection by ascospores of *Penturia inaequalis* (Cke.) Wint. in the greenhouse under controlled conditions; lime-sulphur (liquid), lime-sulphur plus lead arsenate, aerated lime-sulphur, sulphur-lead arsenate dust (90-10) and a commercial colloidal sulphur with and without lead arsenate. All these materials controlled the disease excellently when applied within 24 hours before inoculation. Considerable differences in effectiveness occurred when fungicides were applied after inoculation. Lime-sulphur, (1-40) plus lead arsenate (1-50) controlled leaf infection when applied after an infection period of 45 hours at 7°, or 30 hours at 24° C. Sulphur-lead arsenate dust (90-10) was much less effective, controlling leaf infection well when applied after a 12-hour infection period at 7° C., but not when applied later.

Lime-sulphur (1-40) plus lead arsenate (1-50) applied to scabbed leaves in the orchard prevented the germination of spores which were present and sharply inhibited production of spores from the treated lesions during a period of nine weeks, notwithstanding the occurrence of a series of rains. Sulphur-lead arsenate dust (90-10) gave like results until the first heavy rain, after which viable spores were produced.

Applications of lime-sulphur and sulphur dust, respectively, to comparatively small marked areas of the upper surface of apple leaves protected the entire upper surface from infection by naturally discharged ascospores.

Factors affecting the fungicidal property of sulphur. H. C. YOUNG and ROBERT WILLIAMS.

A part of this investigation is a continuation of the former work on the fungicidal property of sulphur. The results show that pentathionic acid is the toxic factor of sulphur. The test for this acid (the ammoniacal silver nitrate test given in Mellor's Modern Inorganic Chemistry) is positive for almost all sulphurs. The test is very simple, the color being first a brown and then slowly changing to black. It is peculiar that some other workers (English) have "failed to get a positive test upon repeated trials." The sulphide ion is the only sulphur compound that might give a positive test and this has been eliminated by other sulphide tests always being negative on ground or flowers of sulphur.

When sulphur is mixed with basic compounds such as sodium hydrate, potassium hydrate, or calcium hydrate, the salt of pentathionic acid which is not toxic to fungus spores is formed. However, as this salt is formed, more acid is produced and a general equilibrium is maintained. When sulphur was freed of its pentathionic acid and then placed in oxygen free Vantieghem cells it was not toxic to the spores of *Sclerotinia cinerea*. Lime added to sulphur reduced the effectiveness against apple scab in the field.

Heterothallism in the rust fungi. J. H. CRAIGIE.

The writer has made an experimental investigation of sex in the rust fungi and has obtained conclusive evidence that *Puccinia graminis* and *P. helianthi* are heterothallic. The sporidia are of two kinds, (+) and (-). A (+) sporidium gives rise to a (+) mycelium and a set of pyenia which produce (+) pycnosporos. A (-) sporidium gives rise to a (-) mycelium and a set of pyenia which produce (-) pycnosporos. When a (+) sporidium and a (-) sporidium are sown close together on a leaf, the (+) and (-) mycelia resulting therefrom intermingle and produce diploid aecia. When (+) pycnosporos are brought into contact with a (-) pycnium, or (-) pycnosporos with a (+) pycnium diploid aecia are produced on the under side of the pustule receiving the pycnosporos within a few days of the transference.

The pycnium is to be regarded not as a spermatogonium, producing non-functional spermatia, but as an active organ which develops either (+) or (-) pycnosporos and attracts flies by means of which the pycnosporos of one sex are carried to the pyenia of another sex.

Further observations on Corticium koleroga (Cke) v. Hohn. FREDERICK A. WOLF.

A recent paper contains an account of the thread-blight caused by *Corticium koleroga*, on citrus, pomaceous, and a variety of other hosts. The subsequent collections of the fungus on an isolated planting of *Citrus* in the Everglades leads to the belief that it is endemic to that section of Florida.

In another locality it was collected on coral tree, *Erythrina cristagalli*, and the stunted growth of the branches indicated that the fungus was present on this tree when it was introduced from Brazil. Privet *Ligustrum vulgare* and a species of climbing rose growing beneath the coral tree were also affected. Grapefruit and orange growing near were not attacked.

In another locality, the native persimmon, *Diospyros virginiana*, the cultivated persimmon, *D. kaki*, and fig, *Ficus carica*, were affected.

The failure of the fungus to spread to nearby plants of the pear, orange, and the grapefruit in nursery rows from an artificially inoculated pear tree indicates that wind borne basidiospores are of little consequence in the spread of the fungus. Little is known about the factors governing the distribution of *Corticium koleroga*.

Some investigations of Aspergilli by serological methods. TAKASHI MATSUMOTO.

Immune sera were prepared from several species of some groups of Aspergilli by means of ten successive intravenous inoculations of rabbits at intervals of about six days. The results obtained with agglutination experiments were rather unsuccessful, but the reactions by means of complement fixation have shown some significant relationships existing in those strains. They reveal a wide variation, but, as expected, the variation occurring between strains within the same species is not generally so wide as that existing between different species. Some results were also obtained by means of precipitation tests, but the complement fixation seems to be more promising for the serological studies of these particular fungi. It should be remembered, however, that the titre of the sera in these groups is much lower than that of ordinary pathogenic bacteria.

The mycorrhizal fungus of Vaccinium. K. D. DOAK.

During a survey of northern Indiana blueberry regions in 1925 and 1926, a non-pathogenic fungus was found associated in different soils with the roots of *Vaccinium corymbosum* Linn., *V. vacillans* Kalm. and *V. pennsylvanicum* Lam. This fungus developed as intracellular glomerulus masses and hyaline strands and a brownish, appressed external growth on roots in contact with organic particles, and produced an endotrophic mycorrhiza of the type previously described in *V. corymbosum*. A fungus resembling *Rhizoctonia* was isolated from fresh roots of *V. corymbosum* and *V. pennsylvanicum* by three methods. The mycelial characters in culture resembled those of the mycelium growing on roots. Fruiting bodies were not produced. The mycorrhiza was artificially produced by inoculating sterile seedlings with this *Rhizoctonia*.

Normal root and stem development did not depend upon the presence of the fungus, but a profuse growth of it around roots of seedlings was not detrimental. Seedlings transferred into unsterilized sphagnum peat and leaf mold grew more rapidly when roots already possessing the fungus were added. Although extensive development of the fungus often occurred in soil near living roots, there was no evidence of direct nutritive symbiosis.

An examination of Fusaria in the herbarium of the Pathological Collections, Bureau of Plant Industry, U. S. Department of Agriculture. C. D. SHERBAKOFF.

Three hundred and sixty one dry *Fusarium* specimens of the herbarium have been examined. Most of the original determinations had to be changed, but only two new species were found. Three new combinations seemed desirable. These were as follows:

Fusarium tumidum n. sp. Syn. *F. sarcochromum* (Desm.) Sacc. Krieger, *Fungi saxonicæ*, 2499; and Sydow, *Mycotheca germanica*, 1797, on heads of *Sarothamnus scoparius*, 1916.—The fungus resembles *F. culmorum* (W. G. Sm.) Sacc. but has much larger conidia (3–5-septate, 35–82 × 10 µ.).

Gibberella quinquesepata n. sp. On *Cannabis sativa*, Pierceton, Indiana, 1915 coll. L. H. Dewey.—Differs from *G. saubinetii* mainly by 5(3)-septate ascospores.

Fusicoccum fraxini (Kabát et Bubák) n. com. Syn. *Fusarium fraxini* Kabát et Bubák. *Fungi imperfecti exsiccati*, 900, on dried branches of *Fraxinus excelsior*, Bohemia, 1910, 1912.

Ramularia carniformis (E. & T.) n. com. Syn. *Fusarium carniforme* E. & T., on *Tripsacum dactyloides*, herbarium S. M. Tracy, Stockwell, Miss., 1900.

Rhabdospora oxydendri (E. & E.) n. comb. Syn. *Fusarium oxydendri* E. and E., N. Am. Fungi, sec. ser. 3493. On bark of *Oxydendrum arboreum*, Nuttallburg, W. Va., 1896.

Two physiological forms of Ustilago striaeformis (Westd.) Niessl. W. H. DAVIS.

Ustilago striaeformis has been reported parasitic on 24 genera and 41 species of grasses. However, the problem of the physiologic specialization of this smut remains unsolved. This investigation was undertaken to determine whether physiologic forms occur in this smut or timothy (*Phleum pratense* L.) and on redtop (*Agrostis palustris* Huds.) Chlamydospores were collected from both hosts at several stations located in three states. The seedlings employed in the inoculations were incubated from seeds collected at five widely-distributed stations. The methods employed were those already described by the writer. The results were based on 15 series of inoculations, both reciprocal and multiple, in the field and greenhouse, during spring, summer, autumn, and winter. Inoculum taken from timothy infected timothy but not redtop, while inoculum removed from redtop infected but not timothy.

There are two physiologic forms of *Ustilago striaeformis* on these hosts: *U. striaeformis* P. F. 1 (which might also be designated as *phlei-pratensis*) which infects timothy and not redtop; *U. striaeformis* P. F. 2 (which might be designated as *agrostis-palustris*) which infects redtop but not timothy.

The occurrence of Aphanomyces cochlioides n. sp. on sugar beets in the United States. CHARLES DRECHSLER.

Isolations of fungi from sugar beet seedlings collected in fields near East Lansing and Saginaw, Michigan, late in June, 1927, revealed a species of *Aphanomyces*, *A. cochlioides* n. sp. among the several parasites causing damping-off and root-rot. In a well-watered experimental plot, it was responsible for more damage than all other organisms combined. The fungus is presumably identical with the form reported on beets from Germany as *A. laevis*. Entirely normal sexual reproductive structures, produced on suitable agar media, exhibit an oospore wall 1.5 to 2.0 μ thick, never 3 to 6 μ as given by Peters. The oogonial wall, unlike the thin envelope figured by DeBary, measures between 1.0 and 2.5 μ in thickness, and, while exhibiting fluctuations in this dimension from point to point, is not sculptured on its inner surface as prominently as in *A. euteiches*. The fungus differs from the pea parasite also in the frequent flat spiral disposition of the antheridial stalk on the oogonium, helicoid involvement of parts described for *A. helicoideus* Minden, and present in a form isolated from oat roots, however, being generally absent. Contact relationship of elements supporting sex organs prevails much as in *A. euteiches* and in the conspicuously larger *A. raphani* Kendrick.

Paragynous antheridia of Phytophthora spp. DELMER COOPER.

The mycelium of *Phytophthora cactorum* (Lebert and Cohn) penetrates the unbroken epidermis of both apples and pears, by pushing apart the cork cells of the lenticles. A suspension of zoospores placed on the uninjured surface of the fruit also causes infection. The mycelium is intercellular in the host tissue and sends haustoria into the cells. As Rose and Lindegren have reported, oogonia and antheridia occur in the infected pear tissue but not in infected apple tissue. Both paragynous and amphigynous antheridia have been reported for this fungus, but in this study only paragynous antheridia have been found. Cultures of *P. erythrosepica* (Pethyb.), which is

described as having only amphigynous antheridia, were obtained from Pethybridge and from the Centraalbureau, Holland, but no amphigynous antheridia were found. *Phytophthora richardiae* (Buisman) and *P. terrestris* (Sherb.), which are described with amphigynous antheridia and a *Phytophthora* sp. from peony, have also been studied. Only paragynous antheridia have been found, and these are usually closely appressed at the base of the oogonia.

Studies on snapdragon rust, Puccinia antirrhini. E. B. MAINS and DOROTHY THOMPSON.

Urediniospores of *Puccinia antirrhini* germinate through a range of 0+ to 26+° C. with optimum germination around 10° C. While germination was obtained in solution of pH 3.6 to 8.0, the optimum was 6.0 to 6.2. While copper dust reduced germination to some extent, sulphur had the greatest fungicidal effect. Slaked lime also greatly reduced germination. A study of the effect of temperature on infection agreed with the results obtained with spore germination. Dusting with sulphur in the field almost completely controlled the rust, undusted plants dying before midsummer. Selections for resistance over a period of four years have resulted in the development of several lines showing marked resistance. While such lines are infected and mycelium developed to a considerable extent, the host cells often die before the rust is able to sporulate.

Studies upon the Fusarium wilt of china aster. L. R. JONES and REGINA S. RIKER.

The parasite *Fusarium congenitans* var. *callistephi* is scarcely distinguishable from that of cabbage yellows, confirming Beach's statements, but reciprocal crosses showed each to be specific. The temperature relations were also very similar, both as determined for agar plate cultures and for disease development in soil temperature tanks ranging at 4° intervals from 12° to 32° C. No external symptoms of the disease appeared at 12° C. although the plants in infested soil weighed slightly less than the controls; wilt appeared at 16° C. and increased rapidly with rising temperature. Aster wilt was therefore favored by a slightly wider range of temperatures at the lower extreme than cabbage yellows. Development of wilt in the field was also accelerated by high temperature, as is cabbage yellows.

Considerable difference as to disease occurrence has been noted in commercial aster plantations on infested soils, both as between varieties and with individual plants of the same variety. Selections aiming to secure disease resistant strains have been conducted for the three seasons. These have included different colors of Giant Branching and Heart of France. The results are promising, the second generation selections showing in 1937 a much higher degree of resistance than did the commercial controls.

Progress report on the condition of bulbs and corms of ornamental plants offered for importation into Canada. F. L. DRAYTON.

Inspection of foreign importations of nursery stock, including bulbs, by officers of the Dominion Department of Agriculture revealed a highly unsatisfactory condition in many shipments. A study of the literature threw very little light on the economic importance of the various troubles, and the want of reliable information necessitated careful investigation into the nature and significance of these injuries. The bulbs and corms examined included the following: tulips, narcissi, hyacinths, iris (bulbous), snowdrops, gladioli for spring planting, *Gladiolus nanus* types for forcing, crocus, and freesias. The injuries were classified according to microscopic characters and cultured. Among the known pathogens observed were *Bacterium marginatum* McC., *Bact. hyacinthi* Wakker, *Septoria gladioli* Pass., *Botrytis tulipae* (Lib.) Hop., *Rhizoctonia tuliparum* (Kleb.) Whet. et J. M. Arthur, a *Sclerotium* sp. causing a dry rot of gladioli, and fre-

quently *Tylenchus dipsaci* Kuhn, one of the common nematodes affecting bulbs. In addition, a number of *Fusaria* and sclerotia-producing fungi, so far unidentified, have been isolated repeatedly from certain types of injuries. In many cases insect injury and improper handling, curing, etc., encouraged the development of saprophytic fungi, mites, etc., resulting in a more or less severe breakdown during storage and transportation.

Penicillium corn rot of gladioli. O. H. ELMER.

An undescribed storage rot of gladiolus corms was frequently observed in Iowa during the last two years. Infected corms develop dry rot lesions that extend to the heart and that finally include the entire corm. The lesions are brown on the outside and dark gray within. Throughout the rotted area, as well as on the surface, numerous grayish spherical sclerotia develop that average about $\frac{3}{4}$ mm. in diameter. Abundant blue conidial masses are frequently produced at the surface, especially under humid conditions on corms not completely mummified.

Laboratory studies have proved the causal organism to be a species of *Penicillium* (a new species which is being described elsewhere as *Penicillium gladioli* according to a letter from Dr. Charles Thom). Pure cultures were repeatedly obtained from marginal tissue plantings and from surface-sterilized sclerotia. On potato dextrose nutrient agar, conidial production is sparse but sclerotia are produced abundantly. Infections of corms in storage were obtained through wounds only. No infections were obtained on the corms of growing plants.

This disease of gladioli was the most common storage rot found in Iowa and was observed on corms of many varieties. The disease was, in addition, found in corm shipments from Minnesota and Indiana.

A corn rot of gladiolus caused by a Penicillium. LUCIA McCULLOCH and CHARLES THOM.

A rot of gladiolus corms due to a sclerotium-forming species of *Penicillium* has been under observation since May, 1926. Both growing and stored corms become infected through even slight wounds. No infections have been secured by inoculations on uninjured corms. The dark brown, moderately porous rot invades the corm tissues rather rapidly at temperatures between 12 and 23° C. Most characteristic is the production of numerous sclerotia which appear both on the surface and in the interior of the attacked corms. The sclerotia are irregularly spherical; 140 to 540 μ in diameter; smooth; cream to light brown in color. On most culture media the fungus produces at room temperature a small area of dull blue green conidia in the center of the colony surrounded by an area of scanty white hyphae and numerous sclerotia in concentric zones. At temperatures above 20° C. the development of the blue-green conidia is very scanty, while at lower temperatures the conidia are abundant. The pathogenicity of the fungus has been proved by inoculation experiments and the connection of the sclerotia with the *Penicillium* has been definitely established. The fungus has been identified from corms grown in such widely separated regions as Holland, New Mexico, Canada, Kansas, and New York.

Mottle-leaf disease of beech. W. HOWARD RANKIN.

A large percentage of the beech in the general vicinity of Philadelphia have been affected for several years by a mottle-leaf disease of unknown cause. The incidence and severity of the disease do not appear to be correlated with site, age, ground water supply, or any atmospheric condition. No fungus or insect which seemed to have any causal relation to the disease has been observed above ground.

Mottle-leaf is apparent when the leaves develop in the spring. Yellow-green to whitish areas of indefinite outline are present, and these areas often die and turn brown. Seedlings and suckers in full shade often show severe symptoms. Borers and winter injury lead to stag-head and eventually death.

To determine if the lack of some mineral element was responsible, 10 typically affected trees were injected May 25-27, 1927. The trees received from 51 to 240 grams of magnesium nitrate and 10 to 47 grams of ferrous sulfate. The solution used contained 25.6 grams of magnesium nitrate and 5 grams of ferrous sulfate to each liter of water. The rate at which the injections were made varied from 3 liters in 2 hours and 45 minutes to 9.4 liters in 1 hour, 20-25 pounds pressure being applied to the solution.

No marked improvement in the chlorotic condition of the treated trees was noted during the summer.

The penetration of furfural in plant tissues. MARY F. HOWE and DONALD CATION.

Technical furfural painted on the stems of tomato plants from which a small area of the epidermis had been removed caused yellowing and flagging of the leaves and a shrivelling of the stems. High concentrations of furfural are markedly toxic to the green living stem and leaf tissue of herbaceous plants.

The rate of penetration of furfural, through the vascular bundles of the tomato stem, is six times as rapid as water. The furfural penetrates woody stems from four to nine times as rapidly as water. The variation in the rapidity of penetration depends largely upon the type of wood.

The amount of furfural absorbed by woody tissues, such as red oak, hard and soft maple, is from one and one-half to twice as much as the amount of water absorbed.

The amount of absorption by weight of varying solutions of furfural diluted with water (up to 7 per cent) gave no conclusive evidence that the water materially decreased the absorptive properties of furfural. The same size blocks of dry hickory, dry red oak, dry apple, and green apple absorbed less water in a given length of time than furfural, creosote, kerosene, and a creosote-furfural mixture.

An Actinomycete the cause of soil rot or pox in sweet potatoes. J. F. ADAMS.

During the past six years, investigations have been conducted on the cause of pox or soil rot of sweet potatoes. The results have shown that a species of *Actinomyces* is the pathogene rather than the fungus *Acrocystis batatas* reported by Halstead in 1890, or the slime mold *Cytospora batata* as reported by Elliott in 1916. This pathogene has been isolated from typical pox lesions as well as demonstrated to be present in the lesions by histological methods. Direct inoculations have shown this Actinomycete to be pathogenic on cut slices of sweet potato, emerging root points from fleshy, primary, and secondary roots, and stems of sprouts. Resulting lesions simulate those occurring under field conditions. This Actinomycete was also pathogenic on slices of white potato, beet, and turnip but gave negative results on carrot and dahlia. The optimum temperature in relation to growth and infection was found to range between 30° and 37° C. Slight growth or infection occurs under conditions of room temperature. Cultural and inoculation studies also included *A. poolensis* and *A. scabies*. Negative results followed with *A. poolensis*, while *A. scabies* was pathogenic on cut slices as well as rootlets of sweet potato and white potato.

Sweet potato stem rot prevented by treating stems and roots with bordeaux mixture.
R. F. POOLE.

Killing of sweet potato plants in large numbers by the stem rot disease caused by *Fusarium batatatis* Wr. is a result of contamination during the interval of pulling and

resetting the plants. The appearance of the disease under natural field plantings was checked by inoculating plants with a pure culture, and the results were found to compare favorably. Inoculated plants were treated with several strengths of chemical disinfectants with the object of finding a substance which would prevent infection without injuring the plant. The susceptible Porto Rico and Yellow Jersey plants were used; the roots and stems were placed in a spore suspension from a three-day transfer, dipped into the chemical, and transplanted immediately in the field and in pots. A number of chemicals afforded no protection, and some caused severe injury to the plant. Others caused slight retardation of infection, while bordeaux mixture 4-4-50, and stronger, prevented infection in the field and greatly retarded the disease in pots. No attempt was made to control the disease when plants were already infected.

Comparison of various disinfectants in the treatment of sweet potatoes for black rot control: A progress report. O. C. BOYD.

Sweet potatoes of the Porto Rico variety were heavily inoculated with conidia, ascospores, and chlamydospores of the black rot fungus *Ceratostomella fimbriata*, then subjected to one of the treatments listed in table 1, after which the roots were dried and bedded. Each treatment included about 60 roots and was represented in 3 replications in the bed, while the uninoculated control included 138 roots in 5 replications, and the inoculated controls 284 in 13 replications. Soil conditions throughout the draw season

TABLE 1.—*The control of sweet potato black rot in the draw bed obtained by treating the seed with various disinfectants*

Treatment	Percentage of draws infected with black rot	Percentage of bedded roots infected with black rot	Average number of lesions per bedded potato
Uninoculated control	None	None	—
Inoculated control	25.8	96.5	3.8
Mercuric chloride 1-1000, 10 min.	16.2	56.0	2.2
do 2-1000, dip	9.2	56.1	2.0
Bayer Dieldust 1-20, 1 min.	18.0	72.4	2.3
Semesan Bel 1-20, dip	9.0	65.5	1.8
Formalin 3 pt.-50 gal., 10 min.; washed	12.2	81.7	2.0
do 5 pt.-50 gal., 1 min.; washed	13.5	62.0	1.6
Semesan 0.3% solution 10 min.	20.3	95.0	3.0
Semesan dust	18.0	70.7	2.6
du Pont No. 6 Bel 1-20, dip	18.9	74.1	2.4
do No. 21 Bel 1-20, dip	21.4	87.7	2.6
do No. 14 Bel 1-20, dip	21.4	91.4	3.4
do No. 2 Bel 1-20, dip	20.7	91.0	3.6
Semesan Jr. dust	23.2	71.7	2.1
Corona Coppercarb dust (20% copper)	9.2	82.1	2.2
Lucas-Kiltone bordo powder, dust (22% copper)	7.1	88.0	2.0
Colloidal sulphur dust (Niagara Sprayer Co.)	14.6	88.0	2.8

were favorable for the occurrence of black rot. The number of draws produced under the various treatments was not consistently related to the amount of black rot on either potatoes or draws. No appreciable injury resulted from any of the disinfectants, as measured by time of emergence of the draws.

Cytological studies of plant tissues affected with mosaic diseases. J. DUFRENOY.

Studies of living tissues, using neutral red in isotonic solution of cane sugar as a vital dye, and of tissues killed by the formalin and potassium bichromate fixative demonstrate that cells in the light areas differ from the normal green ones as follows: (a) the vacuoles do not stain readily with neutral red; and (b) mitochondria and plastids assume a swollen appearance, so that the chloroplasts, which show in the normal cell as a black network imbedding starch grains, look in the affected cells as if they were converted into spheres which stain a light grey with iron haematoxylin.

Monochromatic light photography in the study of mosaic diseases. FRANCIS O. HOLMES.

Monochromatic ultraviolet light photographs have been made of juices from plants having the following diseases: yellows of aster, strongly mottled mosaic of tobacco, ring spot of tobacco, rugose mosaic of potato, aucuba mosaic of potato, witches' broom of potato, and leaf roll of potato. No formed elements other than those found in normal plants were seen in the pictures of these representative viruses, although excellent resolution was obtained, and over six hundred photographs made.

During the past three years a cytological study has been made of the large intracellular bodies characteristic of Hippenstrum mosaic. It was hoped that they might be identified or at least better understood. The search for an included nucleus or nuclear material was unsuccessful. Chondriosomes were found distributed evenly through the mass of the intracellular bodies just as they are to be found in the host cell cytoplasm. This seemed to indicate that the inclusion is composed of living cytoplasm, whether native or foreign with respect to the host. Photographs of the inclusions with monochromatic blue light showed a characteristic granular structure in the mass of the intracellular body. This could also be seen in the living material.

Experiments with tomato streak. W. G. STOVER.

Streak has been induced by inoculating tomato plants with extract from mosaic tomato together with extract from potato plants with mild mosaic, rugose mosaic, leaf roll, or spindle tuber, and in some cases from apparently healthy potato plants. Streak was not induced when normal seedling potato plants were used as one source of inoculum. Streak was induced in tomato, however, if the potato seedlings had been previously inoculated with potato mosaic. Streak was also induced by inoculating tomato with juice from mosaic tomatoes together with juice from either tomato or black nightshade which had previously been inoculated with potato mosaic.

Streak is transmissible from streak-infected tomato through either tobacco or black nightshade back to tomato. Streak also developed in tomato plants inoculated from either tobacco or black nightshade which had previously been double inoculated with tomato mosaic and potato rugose mosaic. Streak developed in tomato plants inoculated with tomato mosaic and potato mosaic on widely separated dates.

The streak virus mixture may occur in any part of the diseased plant or may be absent from certain organs. The potato element becomes non-infective in a short time after the death of the affected tissue, while the tomato element may remain infective for some time.

Soil transmission of tomato mosaic and streak in the greenhouse. S. P. DOOLITTLE.

It has been found that the viruses of both tomato mosaic and streak will live for at least 70 days in greenhouse soils. These experiments were conducted in a house where both diseases were prevalent during the summer of 1927, using plants which had been grown in sterilized soil at another point. The plants were transplanted under cheesecloth cages in the house in question, the cages being without openings. Control plants on mosaic-free soil remained healthy. Infection occurred in all trials made to date and the experiments are being continued. In the field it has been found that the virus of tomato mosaic will live four to six weeks in the soil, but up to the present there has been no evidence of the overwintering of the virus in experiments where plants are protected by cages. Preliminary studies indicate that the streak of tomato produced by a mixed infection with the tobacco mosaic virus and either mosaic or healthy potato juices is not identical with much of the streak which is found occurring in the field and greenhouse.

Progress on experimental work with the transmission of bean mosaic. T. G. FAJARDO.

Artificial transmission. A modified leaf mutilation method has yielded from 80 to 100 per cent mosaic infection.

Insect transmission. Field and greenhouse cage experiments during 1926 and 1927 gave conclusive evidence of insect transmission of bean mosaic. Successful results were obtained with three species of mosaic-reared aphids and mealy-bugs. Negative results have thus far been obtained with leafhoppers, 12-spotted and striped cucumber beetles, red spider, thrips, tarnished plant bug, and white fly.

Soil transmission. Contact of roots on aerial parts and picking pods simultaneously between mosaic and healthy plants under cages failed to cause mosaic infection; transmission through soil likewise failed.

Seed transmission. Bean mosaic commonly overwinters in infected seed and in no other way thus far determined, no other hosts having been found. The percentage of seed infection varies widely with the variety, as high as 50 per cent being found in commercial seed of susceptible varieties. Plants grown from infected seed yielded in turn a higher percentage of infected seed than plants inoculated during their vegetative development, but in case of blossoming plants no infection of seed resulted in pods which had set previous to inoculation. On uniformly diseased mosaic plants there was less infection in late than in early set pods.

Virus diseases observed by the Allison V. Armour Expedition. H. H. MCKINNEY.

On the Canary Islands, mosaic was very prevalent on wild *Nicotiana glauca*. Three types of mosaic symptoms, a dark green, a light green, and a yellow, were found on this species. The light green type, which seems to be identical with the mosaic generally found on tobacco in the United States, was the most prevalent. Studies of these viruses on tobacco show that the yellow type can be isolated from yellow spots which are associated with the light green type. The dark green type shows excessive chlorophyll production, and the mottled areas are unusually large but few in number. Mosaic was found also on *Psoralea bituminosa*, wild garlic, and Irish potatoes.

In West Africa, mosaic is general on *Capsicum* sp. and on *Manihot esculenta*, which is an important fruit plant. In some cases mosaic seemed to be a limiting factor in its production. Yellow and green types of mosaic were found on three species of Cucurbitaceae and on several other plants.

Wild grasses were flourishing in the Cameroons, and sugar-cane was growing in all the Colonies visited, but no grass mosaic was found.

Further studies on the host range of aster yellows. L. O. KUNKEL.

During the past year the known host range of aster yellows has been extended by the experimental transmission of the disease to 21 species in 13 different families of plants. Transmission was accomplished in all cases by means of the leafhopper, *Cicadula sexnotata* Fall. Most of the new hosts are cultivated flowering plants. Aster yellows was carried to plants in five families from which susceptible species have not previously been reported. The disease has now been experimentally transmitted to more than 70 species in 28 different families of plants. Although aster yellows has a very wide host range, many species closely related to susceptible ones are immune. *Plantago major* L. is susceptible, but *P. lanceolata* L., though one of the favorite host plants of the aster leafhopper, is immune. *Centaurea imperialis* Hort. is one of the most susceptible species, but *C. cyanus* L. does not become infected.

It has been demonstrated by transmission experiments that aster yellows is distinct from witches' broom of potatoes and from a yellows disease of boneset (*Eupatorium perfoliatum* L.) which is widely distributed throughout the Middle West.

Further studies on the attenuation of plant viruses. JAMES JOHNSON.

The attenuation of the ordinary tobacco mosaic virus by the exposure of newly inoculated plants to a constant temperature of 35–37° C. for 10 days has been previously reported. Continued studies on this subject show that little or no observable attenuation can be secured at temperatures below 34° C. with an exposure of 15 days. At this temperature a milder degree of attenuation was secured than can be obtained with a 15-day exposure at 38° C. Some of these attenuated viruses have remained attenuated after ten serial transfers through tobacco or after storing *in vitro* for several months. The tobacco mosaic virus has also been attenuated by means of bubbling air and oxygen through the virus extract for several weeks. The same virus has also been secured in a naturally attenuated form from several soils in which it had overwintered. The cucumber mosaic virus on tobacco has also been attenuated by exposure to a temperature of 37° C. for 10 days. Attenuation of viruses is rather sporadic and may not be secured in every case by any of these methods. Attempts to attenuate the potato rugose mosaic virus in a similar manner were unsuccessful.

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THE CONTROL OF TELIOSPORE AND UREDINIOSPORE FORMATION BY EXPERIMENTAL METHODS¹

CHARLES W. WATERS

INTRODUCTION

The significance to be attached to the formation of teliospores² following production of urediniospores in the rust-fungi has been problematical ever since this very important group of parasitic fungi has been investigated by botanists. The assumption that it is an innate characteristic which must find its expression during the course which the fungus takes in its development in the host has long been shown to be insecure, not only by common observation but by studies on the subject such as those attempted by Iwanoff (18), Morgenthaler (28), Gassner (14), Smith (40), and others.

These investigators have attempted to show by experimentation and observation that the telial generation of the rusts stands as an expression of the ability of the fungus-parasite to react to certain environmental conditions unfavorable for its continued vegetative development and to produce a fruiting structure which will tide the fungus over such adverse conditions.

Among the various causes that have been suggested as stimulating the production of such a resistant stage are direct climatic influence, temperature, moisture, resistance of the host, inner organization of the fungus, indirect climatic influences acting through the host, and so forth. However, in practically all of the experiments and observations that have been made in the past, there has been no serious effort to get at the bottom of the problem and attempt, by correlation of the results which the various factors produce, to deduce some fundamental principle which might underlie and govern the appearance or non-appearance of the teliospore generation in the rusts.

It has been the aim of this work to determine such an underlying principle and, in this paper, to analyze the results obtained and their relation to such a principle.

¹ Papers from the Department of Botany of the University of Michigan, No. 271.

² Since the terminology of the spore forms as suggested by Arthur (4) is gradually replacing the old, the former will be used throughout the paper.

The work was done in the Cryptogamic Laboratory of the University of Michigan under the direction of Professor C. H. Kauffman, to whom the writer is greatly indebted for generous advice and helpful criticism.

EXPERIMENTAL RESULTS

In the following work ten species of rusts were used: *Uromyces appendiculatus* Fr., on *Phaseolus vulgaris* L.; *Puccinia taraxaci* Plowr., on *Taraxacum officinale* Weber.; *Puccinia suaveolens* Rostk., on *Cirsium arvense* (L.) Scop.; *Puccinia sorghi* Schw., on *Zea mays* L.; *Puccinia asparagi* DC., on *Asparagus officinalis* L.; *Puccinia triticea* Eriksson, on *Triticum vulgare* Vill.; *Uromyces trifolii* Lev., on *Trifolium hybridum* L.; *Puccinia antirrhini* Diet. and Holw., on *Antirrhinum majus* L.; *Uromyces polygoni* Fuckel, on *Polygonum aviculare* L.; and *Puccinia orbicula* Pk. and Clint., on *Prenanthes alba* L.

Experiments were conducted with these rusts both on the hosts grown in pots in a greenhouse and on leaves or portions of leaves of the hosts grown on solutions in petri dishes in the laboratory.

Technique

In the Greenhouse

In all cases when the healthy host plants were brought in from the field and infection was obtained, checks were maintained and remained healthy. The various hosts were cultivated in pots in the greenhouse and infection obtained in a manner similar to that suggested by Melhus (26) and Fromme (12), that is, spore suspensions were made in distilled water and sprayed on by means of a No. 16 De Vilbiss atomizer, after which the plants were placed in a moist chamber for 48 hours at a temperature varying from 17° to 22° C., depending on the rust. Variations of this method were used at times, such as first spraying the plants with distilled water and then applying the inoculum with a scalpel or camel's-hair brush as advocated by Carleton (7). The latter method insures a heavier infection, but if one desires a uniform infection for comparative studies the former method is the more satisfactory.

In working with plants such as alsike, wheat, or corn, the waxy coating of the leaves prevents the spore suspension from adhering. In such cases it is necessary to draw the leaf carefully between the moistened thumb and fore-finger before applying the suspension (Melchers, 25).

In the greenhouse, various incubation chambers have been employed. Under the ordinary conditions met with during the greater part of the year, a Wardian case and bell jars have been found to be the most satisfactory. In the summer, however, it was found to be almost impossible to infect the

plants successfully by these means alone, due to the prevailing high temperatures. The "iceless refrigerator," as suggested by Hunt (17), solved the difficulty. In the refrigerator, with modifications, infection was obtained with as much ease in the hottest part of the summer as at any other time of the year. It has been found by the writer that, on a day when the temperature registered 35° C. in the greenhouse, by means of the "iceless refrigerator," it was possible to keep the plants at a temperature not exceeding 26° C. This was accomplished by opening the ventilators of the greenhouse in such a way that a breeze was constantly blowing over the surface of the refrigerator, thus increasing the evaporation and lowering the temperature. It was found, by constructing the refrigerator of sufficient size to enclose the Wardian case, that the humidity was kept higher and the plants became more heavily infected than when they were left uncovered in the refrigerator. During the spring and fall of the year when the temperature was not sufficiently high to warrant the use of the refrigerator, a metal pan was used as a cover for the Wardian case. This was kept filled with water and lowered the temperature of the case sufficiently for infection.

In applying the inoculum to the leaves of the various hosts, a difference was found in the amount of infection produced, depending upon which surface received the spores. As the stomata are, so far as we know (Ward, 46) (Evans, 11), the only means of entrance for the germ tubes of the urediniospores, the amount of infection resulting from the inoculation will vary directly with the number of stomata.

In working with infection on hosts such as corn and wheat, in which the leaves assume a more or less vertical position, the writer has been unable to find a correlation between the amount of infection and the surface receiving the inoculum, although Melchers (25) reports one-half as many stomata on the lower surface of wheat leaves as upon the upper. However, on hosts such as bean, alsike, snapdragon, etc., it was found that more severe infection was produced by inoculation on the lower surface. The snapdragon shows the greatest degree of variation, especially when leaves were inoculated in petri dishes. In the first part of the work the snapdragon leaves were always inoculated with the upper surface uppermost. The result was a very slight infection. Finally, leaves were placed in the dishes in a reversed position and the spores applied to the lower surface. Two days later the position of the leaves was changed, so as to increase photosynthesis, and just before the time for the uredinia to break through the epidermis they were again placed in the position which they occupied at the time of inoculation. Heavy infection resulted from such procedure. In all subsequent inoculations of snapdragon leaves in petri dishes, therefore, the spores were applied to the lower surface. The ideal arrangement for producing infection in petri dishes would be to place the leaves with their lower surfaces

up and have the light enter from below. This was tried by means of electric lights with noticeably increased infection. But, owing to the difficulties attending such an arrangement, it was not employed in any of the recorded experiments.

In working with plants in the greenhouse during the period of short days, the light factor became increasingly more difficult to control. With plants such as corn, beans, snapdragon, and wheat, no special difficulty was encountered. With the other hosts, however, such as alsike, Canada thistle, *Taraxacum*, and *Polygonum*, during the short days of December and January, it was almost impossible to maintain the rusts. It was only through the added influence of electric lamps, suspended a few feet above the pots, that the writer was able to keep the last-named rusts in a condition suitable for experimentation.

In addition to the difficulties that were encountered with respect to light, there arose other factors that were detrimental to the best development of the rusts. Powdery mildew made its appearance from time to time on the dandelion, alsike, wheat, bean, and *Polygonum*, and was only with difficulty controlled. Once a plant became infected with rust it was almost impossible to eliminate mildew without also killing the rust. However, by inoculating the plants with the rust and then allowing several days to elapse to insure entrance of the germ tubes, the leaves could be safely sprayed with a 1-1000 aqueous solution of sulfuric acid. The plants were later rinsed with water, and by the time the uredinia broke through the epidermis the leaf was practically free from mildew. Fromme (12) reports using this method with sulfur dust 24 days after inoculation.

In addition, insect pests added other difficulties. With the advent of the various leaf hoppers, white flies, thrips, and red spiders, the rusts at times encountered almost overwhelming competition. As it is an established fact that a vigorous host is necessary for a successful rust infection, it is essential in working with the rusts to have a good supply of healthy vigorous host plants. To obtain them, however, is not an easy matter in the average greenhouse.

In Petri Dishes

In inoculating leaves in petri dishes, the same methods were used as in the pot inoculations. In preparing leaves for inoculation, care was taken to reduce to a minimum contamination from extraneous fungi.

The leaves were first removed from the host plant and immediately placed in a dish of water. They were then washed for several minutes in running water, rinsed in several changes of distilled water, and finally placed in a dish of sterile distilled water until ready for use.

The petri dishes were sterilized in the hot-air oven in the usual manner and the leaves placed therein. In the early experiments the solutions were

always placed in the dishes at the same time as the leaves. Later experiments proved, however, that better infection resulted if the spores were sprayed on the leaves and just enough water added to the dishes to keep the air saturated for 48 hours; at the end of this time the solutions were added and the petioles of the leaves cut back in order to facilitate intake of the nutrient.

Various methods of placing the leaves in the dishes were tried out. The first method used was to place glass rods in the bottom of the dishes and allow the leaves to rest upon them. The solution was then added in such amounts that the leaves did not come in contact with the solution except for the immersion of the petiole. Another method tried was to cut circular discs of screen wire with the edges bent down in order to raise them to the proper height for the dishes and then dip them in melted paraffine to prevent rusting. The leaves were laid upon these wire discs with the petioles extending down through one of the openings into the solution. With these methods, leathery leaves became infected, but tender leaves usually died before infection took place.

Clinton and McCormick (8) used a method somewhat similar to this: rubber bands were stretched across the dish and the leaves placed upon them. These investigators encountered the same difficulties as the writer in attempting to infect delicate leaves.

The method finally used was to float the leaves freely upon the solution either sugar or distilled water. No leaf has yet been tried which could not be kept alive and in good condition for five or six weeks with this method. When floating leaves on sugar solutions, from which the leaf is to absorb nourishment, the more cut surfaces exposed to the solution the greater the amount and the more rapid will be the absorption. This can be easily demonstrated by clearing the leaves and applying the iodine test for starch, for it is this form of carbohydrate into which the sugar is transformed for storage in most leaves. This has been shown very beautifully by Saposhnikoff (35), Aeton (1), Tollenaar (44), and others. It appears that the starch is deposited centrifugally from the vascular bundles outward, the more distant cells showing the starch last. When the cut surfaces are exposed the sugar apparently travels from cell to cell by diffusion but more slowly than through the bundles. Various nutrient solutions were used upon which to float the leaves, but it was found that a five to seven per cent sucrose solution gave the best results. Ordinary commercial cane sugar was used in all the experiments, and no attempt was made to secure a chemically pure product. It was found in the course of the work that not only did the sucrose solution prove to be the form of nutrient that was best utilized by the various leaves, but it remained uncontaminated for a much longer period of time. Especially was this true of the unsterilized solution.

At first, care was taken to sterilize the solution of sucrose before it was poured into the dishes, but experience soon showed that the unsterilized solution remained free from contaminating fungi for a longer time than that which had been sterilized. This is due, possibly, to the hydrolysis of the sucrose during the sterilization process, the resulting monosaccharides offering more ready food to the contaminating fungi. Mudge (29), however, claims that practically no sucrose is inverted in an aqueous solution during sterilization. He sterilized solutions for as long as one hour under 15 pounds pressure and found practically no inversion. If this be true, the writer is at a loss to explain the uniform results obtained, unless they were due to the slight impurities in the commercial cane sugar used.

The ability of leaves and other portions of green plants to take up carbohydrates from solutions upon which they are floated has been observed by various investigators, among whom are Boehm (6), Meyer (27), Acton (1), Saposchnikoff (35, 36), Parkin (32), Atkins (5), Mains (21), and Tolenaar (41). Almost without exception they have found sucrose to be the most readily utilized of any of the sugars. They found that the optimum concentration range is from 4 to 20 per cent, depending on the conditions and type of leaf.

Among the nutrients tried during the present work were glucose, fructose, starch, maltose, raffinose, asparagin, malt extract, peptone, and cane sugar. In addition, Shive's (39) three-salt solution was added to various solutions, but the results showed that for all purposes of rust infection a simple solution of commercial cane sugar was the most satisfactory.

When leaves are kept in sugar solution, light is not necessary during storage, as the leaves absorb carbohydrates directly from the solution. Experiments, however, have shown that contamination by fungi is materially lowered when leaves in sugar solution are placed in diffused light instead of total darkness. At first they were kept under a black screen in the greenhouse, but during later experimental work they were kept on the same table as the leaves in the distilled water. In this way the leaves received the benefit of the carbohydrate solution plus any photosynthesis that may have been carried on.

The dishes containing the leaves upon distilled water had to be placed in a lighted place, as the rust-fungus obtains its nourishment only from the food which is stored in the leaf prior to inoculation or from the photosynthetic products formed in the leaf while in the dish. It was found, however, that if they were placed in direct sunlight the leaves were rapidly scalded. The plan finally used was to place them on a table in the north window where they received sufficient light to insure infection.

During the winter months when the days were short and the light in-

tensity low, electric lamps were suspended above the dishes. With the aid of these, infection was maintained satisfactorily.

The tables upon which the dishes were kept were washed thoroughly every other day with a two per cent solution of "Neko," a carbolic acid preparation (Parke Davis and Co.), to keep contamination at a minimum. The floor of the room was also washed with "Neko" occasionally. It was necessary to change the solutions in the dishes several times a week. The leaves were removed from the dishes, carefully washed in sterile distilled water, the dishes rinsed, and the leaves replaced in new solutions. If the petiole or the cut edges of the leaves appeared to be attacked by bacteria or molds, they were trimmed back so as to allow the continued entrance of the solution. The mortality rate with dish cultures is necessarily high because the leaf tissues are often bruised in handling. However, with careful observance of the rules of culture technique, a large percentage of the inoculated plants became infected. In practically all cases infection was more easily obtained on the leaves or portions of leaves in the petri dishes than on the natural host in the greenhouse.

Greenhouse Experiments with Potted Plants

The experiments given below have been grouped as far as possible according to the reactions of both host and parasite to certain environmental factors, such as light, temperature, moisture, and the resistance of the host. The influence of the food and chemical factors comes essentially from the host. As the temperature and light experiments were conducted at the same time, they will be grouped together.

• Temperature and Light

The response of the host and parasite to these two environmental factors, both singly and combined, were investigated in nine rusts. They will be taken up in the order of experimentation.

Uromyces appendiculatus Fr., on *Phaseolus vulgaris* L. (variety: *Tennessee Green Pod*). In Experiment 1, twenty pots of bean were inoculated by spraying with a spore suspension and then placed in the moist chamber for 48 hours at a temperature of about 20° C. At the end of this time they were placed in the greenhouse and allowed to remain until urediniospores were formed. On the eighth day after inoculation, just as the uredinial pustules were breaking through the epidermis of the leaves, five pots were removed to a dark room at 19° C. and allowed to remain for two days. Five pots were also placed in a cold dark room at 7° C. and removed at the end of two days. The remaining ten pots were left in the greenhouse under ordinary conditions at 19–25° C. The results are shown in table 1.

TABLE 1.—*The effects of temperature and varying amounts of light on time of teliospore formation in Uromyces appendiculatus on Tennessee green pod beans*

Experiment 1

No. plants	Incubation period in days	Treatment after 8-day incubation period ^a	Formation of telio- spores (no. days after inoculation)
5	8	In dark room, 19° C. for 2 days	17
4	8	In dark room, 7° C. for 2 days	15
9	8	Checks, in greenhouse at 20° C.	28

Experiment 2

No. plants	Incubation period in days	Treatment 4 days after inoculation	Formation of telio- spores (no. days after inoculation)
5	11	In dark room, 19° C. for 4 days	12
4	12	In dark room, 7° C. for 2 days	13
4	8	Checks, in greenhouse at 20° C.	26

Experiment 3

No. plants	Incubation period in days	Treatment 4 days after inoculation	Formation of telio- spores (no. days after inoculation)
3	11	In light, 7° C. for 2 days	13
3	11	In dark room, 7° C. for 1 day	13
4	12	In dark room, 19° C. for 4 days	13
4	8	Checks, in greenhouse at 20° C.	24

Experiment 4

No. plants	Incubation period in days	Treatment after 9-day incubation period ^a	Formation of telio- spores (no. days after inoculation)
5	9	In light, 7° C. for 2 days	19
4	9	In dark room, 7° C. for 2 days	17
4	9	In dark room, 19° C. for 2 days	24
5	9	Checks, in greenhouse at 20° C.	28

^a Urediniospores had formed at this time.

In explanation it may be said that under ordinary conditions of infection of bean leaves, secondary and tertiary circles of uredinia form about the primary sorus before the telial stage arises. When teliospores are formed, they usually appear in the two later-formed circles of pustules, seldom in the primary. In the above experiment, on those plants which were placed in the cold dark room, there was about 100 per cent teliospore production in the primary pustules at the end of the fifteenth day; on those which had been placed in the dark room at 19° C., there was 8 per cent teliospore production in the primary pustules; and in the case of the checks the teliospores were interspersed with the urediniospores in the secondary and tertiary pustules, with none in the primary. In the two former cases practically no secondary or tertiary pustules had formed.

These results show that both darkness and low temperature tend to suppress the uredinial stage and to favor the early production of teliospores.

In Experiment 2, the plants were removed to the dark rooms four days after inoculation to note the effect on the incubation time and also on time of teliospore formation. The results of this experiment are summarized in table 1, Experiment 2.

From the results of Experiment 2 it is seen that the incubation time is lengthened by approximately the time that the plants are kept in darkness and at low temperature.

That metabolism is not completely inhibited at 7° C. is shown by Experiment 3, in which the plants were placed in darkness at 19° C., in darkness at 7° C., and in a room at 7° C. which received light approximately 15 hours a day. In all these cases the plants were placed under the several conditions four days after inoculation. Results of Experiment 3 are summarized in table 1.

On all the plants with the exception of the checks, teliospores were found interspersed with urediniospores at the end of the incubation period.

It is apparent that light even at as low a temperature as 7° C. exerts a favorable, indirect effect upon the fungus, for the incubation period in this experiment was the same as that of the rust which was in the dark at 7° C. for only one-half the time.

In Experiment 4 the same conditions of temperature and light were used as in Experiment 3, except that the plants were placed under the changed environmental conditions at the time of the first appearance of uredinia, *i.e.*, nine days after inoculation. The results of this experiment show a relative delay in the formation of the spore forms. The incubation period was longer than usual, and the telial stage appeared later. However, the comparative results remained the same.

In the experiments thus far conducted, inoculations on the bean were always made upon the first two leaves that appeared, namely, the primordial leaves. As practically all the photosynthesis that takes place at this young stage of the plant is carried on in the primordial leaves, there is a constant withdrawal of a portion of such elaborated food for the development of the later foliage, the trifoliate leaves. By clipping back the later developing shoots it should be possible to retain a large part of this food in the primordial leaves. Thus if teliospore formation is an indication of a famished condition in the host plant, those plants that were clipped should show a comparatively longer period of urediniospore production than those that were allowed to maintain their normal growth. Accordingly, experiments were conducted to determine if this hypothesis were true.

In the next experiment, therefore, one-half of the inoculated plants were clipped and the other half allowed to retain the normal habit of growth. All plants were placed under the same environmental conditions. On those plants which had been infected and clipped, teliospores were produced from 32 to 34 days after inoculation, while on the unclipped plants the telial stage was produced from 26 to 28 days after inoculation.

The primordial leaves on the unclipped plants presented a decidedly different appearance than did those on the clipped plants. They were of a paler color and less leathery in texture, and at the time of teliospore formation showed the effects of the rust to a much greater degree than the others (Fig. 1). The sori upon the leaves of the unclipped plants were smaller, there were fewer secondary sori, and in general the leaves seemed to contain less food than those upon the plants which were clipped. The fact that the telial stage appeared on the unclipped plants from six to ten days earlier than on the clipped plants seems significant enough.

The comparative effects of temperature and light on the clipped and unclipped plants were determined by placing the plants in the several environments at the time of uredinial formation. The results are given in table 2.

TABLE 2.—*The effect of temperature and light on time of teliospore formation in Uromyces appendiculatus on "clipped" and "unclipped" plants of Tennessee Green Pod beans*

Plant no. and condition	Treatment after 7 to 8-day incubation period	Formation of telial stage	
		No. days after inoculation	Remarks
B 87-90 clipped ^a	In dark room, 7° C., for 2 days	14	In primary and secondary pustules.
B 91-93 unclipped	In dark room, 7° C., for 2 days	14-15	In primary pustules. No secondary ones found.
B 94-96 clipped	In dark room, 19° C., for 2 days	15-17	In secondary pustules.
B 97-100 unclipped	In dark room, 19° C., for 2 days	15-17	In primary pustules.
B 101-110 clipped	Checks, in greenhouse at 20° C.	30	In secondary pustules.
B 111-115 unclipped	Checks, in greenhouse at 20° C.	26	In secondary pustules.

^a Plants with growing point removed.

These results show that, when the plants are placed in darkness at either room temperature or reduced temperature, the formation of telia is not hastened appreciably by removal of the growing point. It is only when the plant is allowed to remain under normal conditions of light and temperature and when metabolism is going on at a normal rate, with a constant increase in size of the trifoliolate leaves, that the drain on the food from the primordial leaves is made apparent by the earlier appearance of the teliospore generation.

Fromme and Wingard (13) assert that in resistant varieties of beans the incubation time may be lengthened and teliospores make their appearance without being preceded by the uredinial generation as is normally the case in susceptible varieties. These results were verified by the writer in experiments conducted under normal conditions and under conditions as outlined above. The Scarlet Wax bean was used as a resistant variety.

Plants of Scarlet Wax and Tennessee Green Pod of the same age were inoculated at the same time. Nine days after inoculation the uredinia broke through the epidermis of the susceptible variety, and three days later they appeared on the resistant one. Primary and secondary sori formed on both varieties but on the susceptible one the sori were larger, more numerous, and continued to produce urediniospores for a longer time than sori on the resistant one. Teliospores appeared on the Scarlet Wax 14 days after inoculation, but it was not until the twenty-third day that they

formed on the Tennessee Green Pod. In the former case they were produced in the primary and secondary sori, while in the latter case they were formed in the secondary only. On some of the plants of the Scarlet Wax variety, teliospores were found interspersed with urediniospores in the very first pustules. In order to note the effect of unfavorable conditions on such

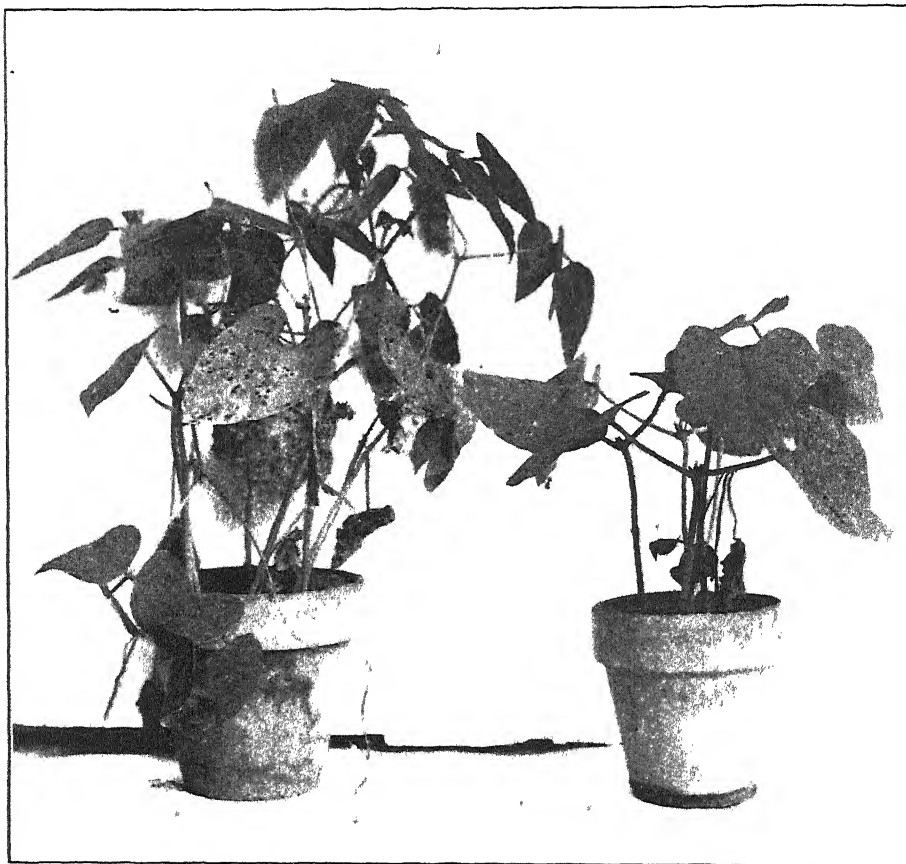


FIG. 1. Rusted bean plants, showing condition of the primordial leaves on plants with and without the growing points removed.

a host during incubation, several plants of Scarlet Wax were inoculated and four days later were placed under different conditions of light and temperatures. Table 3 gives the results of this experiment.

From the results it is evident that, when resistant varieties of beans are infected with the rust, there is a tendency for the uredinial generation to be shortened or even suppressed. When plants are placed under unfavor-

TABLE 3.—*The effect of temperature and light on time of teliospore formation in Uromyces appendiculatus on Scarlet Wax beans*

No. plants	Incubation period in days	Treatment 4 days after inoculation	Formation of teliospores	
			No. days after inoculation	Remarks
7	11	In dark room, 7° C., for 1 day	11	In primary pustules.
4	14	In dark room, 19° C., for 4 days	14	In primary pustules.
7	15	Checks, in greenhouse at 20° C.	15	In primary and secondary pustules.

able conditions during part of the incubation period, the uredinial generation may be eliminated entirely.

Puccinia suaveolens Rostr., on *Cirsium arvense* (L.) Scop. *Cirsium* rust, in nature, forms an abundance of teliospores throughout the growing season. Teliospores may be found on badly infected plants at all times, even in the primary systemic infections early in the summer.

Rostrup (34) and Olive (30) showed that *Puccinia suaveolens* was systemic and that the mycelium hibernates in the rhizome. Plants in the field infected with the primary stage of the rust never blossom, and they can be readily detected by their pale, spindly appearance. Adjacent plants are infected by the primary urediniospores which give rise to local infection, much less damaging in its effect.

For experimental material of this rust, plants were brought in from the field and planted in pots in the greenhouse. After becoming adapted to their new environment they were inoculated by spraying with a spore suspension, the spores of which were obtained from infected plants collected in the field. The incubation period of this rust varies from six to ten days, the optimum temperature for germination being about 18°–22° C.

The uredinia appear almost always on the upper surface of the leaf, although the primary uredinia usually appear on the lower. Later the sori may extend through to both surfaces. Under optimum conditions of temperature and moisture the rust may not produce teliospores for more than a month after infection. In most cases, however, the uredinia are found to contain teliospores as early as two weeks after infection. The teliospores appear on the lower leaves first and then extend progressively up the plant. This rust will not propagate itself in the greenhouse but must be re-inoculated about every 30 days in order to keep it in the uredinial stage. Under conditions of high temperature and low humidity the regions surrounding the uredinia will become dry and the rust will die.

The first experiment with this rust was conducted in order to note the effects of temperature and light on the type of spore that would be produced.

Twenty potted plants were inoculated and placed under various conditions of light and temperature similar to those used in the experiments with the bean. The results are shown in table 4.

TABLE 4.—*The effect of temperature and light on the time of teliospore formation in Puccinia suaveolens on Cirsium arvense*

No. plants	Incubation period in days	Treatment after 7-day incubation period	Formation of teliospores (no. days after inoculation)
5	7	In dark room, 19° C., 5 days	28
6	7	In dark room, 7° C., 5 days	24
10	7	Checks, in greenhouse at 20° C.	32

These results show that telial formation can be hastened by subjecting the infected plant to conditions unfavorable for metabolism in the host. When the plants were removed from the dark rooms, the areas surrounding the pustules were lighter than the remainder of the leaves and on some of the plants these areas were completely dried up.

In the next experiment an attempt was made to induce the formation of teliospores by removing the plants from pots, immersing their roots in distilled water, and placing them in the dark at a temperature of 19° C. Plants C20-25 and C25-30 were removed from the pots of earth at the time of uredinial formation and placed in flasks of distilled water, the roots being carefully washed of all particles of soil. Here they remained for two days in the sunlight, after which they were placed in the dark room for two days. At the end of this time plants C20-25 were placed in flasks of Shive's nutrient solution and returned to the sunlight, while plants C25-30 were left in distilled water and returned to the sunlight. Plants C30-35 and C35-40 were left in pots during the entire experiment (Table 5).

On plants C20-25, which were transferred at the time of uredinial formation to distilled water for two days, and then placed in the dark room for two days and finally transferred to Shive's solution for the remainder of the time, teliospores appeared on the sixteenth day after inoculation.

On plants C25-30, which were placed in distilled water at the same time as C20-25, allowed to remain for two days, and transferred to the dark

TABLE 5.—*The effect of light and nutrient solution on teliospore formation in Puccinia suaveolens on Cirsium arvense*

Plant no.	Treatment after 6-day incubation period	Formation of teliospores	
		No. of days after inoculation	Remarks
C20-25	Removed from pots and placed in distilled H ₂ O in light for 2 days, then in darkness for 2 days; transferred to Shive's solution and placed in light	16	Teliospores numerous.
C25-30	Do ; left in distilled H ₂ O, not Shive's solution	—	Pustules dried up. No teliospores.
C30-35	Potted plants in dark for 2 days, then returned to light	25	Teliospores numerous.
C35-40	Cheeks, in greenhouse	32	Teliospores mostly on lower leaves.

room for two days, there was no telial formation whatever. The portions of the leaves around the rust pustules dried up and a few days after removal from the dark room the rust had apparently died. The remaining plants, C30-35 and C35-40, were accorded the same treatment as plants in the earlier experiment, not being transferred from pots, and the results were practically the same.

Plants C20-25 continued to produce new leaves in the Shive's solution for 60 days after the transfer, the rusted leaves slowly drying up and dropping off, leaving the plants free from rust.

Results of other experiments similar to these were the same; in all cases the placing of infected plants in the dark either at low or room temperature was sufficient to inhibit further formation of urediniospores. In some cases, if the plants were not allowed to remain under such conditions for too long a period, uredinial production was resumed in the same pustules after teliospores had been produced. Especially noticeable was this in *Uromyces polygoni*, *Puccinia antirrhini*, and *Puccinia taraxaci*.

During winter days when the light intensity was low and the hours of daylight few, it was very difficult to maintain a stock supply of urediniospores of *Puccinia suaveolens*. When plants were infected, in many cases the first pustules that broke through contained 50 per cent of teliospores. In no case was it possible to maintain the uredinial stage for more than two weeks after the first infection. This difficulty was partially overcome by installing seven 100-watt electric lamps in the experimental room of the greenhouse. These were suspended about three feet above the pots and were supplied with reflectors to concentrate the rays of light. With the

aid of this auxiliary illumination, which was turned on from about four o'clock in the afternoon until eleven at night, it was possible to secure infection and prolong the uredinial stage to three weeks or more.

Uromyces trifolii Lev. on *Trifolium hybridum* L. Plants of alsike were grown from seed and inoculations made in the usual manner. The incubation time of this rust varies from 7 to 10 days, the optimum temperature for spore germination being about 18–20° C. The pustules are first evident as brownish areas on the under side of the leaves, the uredinia first breaking through on that surface, then later extending through to both surfaces. Under ordinary conditions in the greenhouse, teliospores are not formed in appreciable quantities, the leaves usually drying up before such a stage is reached. Occasionally they are formed on the petioles of the older infected leaves, and in very rare cases upon the blades, interspersed with urediniospores. This condition of course will vary, depending upon the amount of infection and environmental conditions.

The first experiment with this rust was to determine the effects of light and temperature upon the type of spore that would be produced.

Young plants, varying from four to five inches in height, were inoculated. At the time of appearance of the uredinia, some were placed in the dark at a temperature of 19° C., and others in the dark at 7° C. The results are shown in table 6.

TABLE 6.—*The effect of light and temperature on time of teliospore formation in Uromyces trifolii on Trifolium hybridum L.*

No. plants	Treatment after 8-day incubation period	Formation of teliospores	
		No. days after inoculation	Remarks
5	In dark room, 7° C., for 4 days.	—	No teliospores formed. Leaves dried up.
4	In dark room, 19° C., for 4 days.	32	Teliospores on petioles only. Few in number.
6	Checks. in greenhouse at 20° C.	46	A few scattered teliospores on petioles.

It is evident that the treatment accorded the plants placed in the cold dark room was too severe to allow the production of any additional spores after the plants were removed. The leaves had completely withered and the plants had not grown after being placed in the dark. The host was too rapidly weakened to support the formation of teliospores. Even in the case of those plants placed in the dark at room temperature, the leaf blades had withered and the few teliospores that had formed were found upon the peti-

oles of the leaves. It was evident from these experiments that some method less drastic must be devised which would extend over a longer period of time in order to avoid killing the host too soon.

In the next experiment the plants were placed under the changed environmental conditions for shorter periods of time (Table 7).

TABLE 7.—*The effect of varying periods of light and temperature on time of teliospore formation in Uromyces trifolii on Trifolium hybridum*

No. plants	Treatment after 8-day incubation period	Formation of teliospores	
		No. days after inoculation	Remarks
5	In dark room, 7° C., for 1 day; in dark room, 19° C., for 1 day; in greenhouse at 20° C. for 3 days; in dark room 7° C., for 1 day.	36	Some telia on leaf blades but mostly on petioles.
2	In dark room, 7° C., for 2 days.	35	Teliospores on petioles.
3	In dark room, 7° C., for 3 days.	29	Few teliospores formed. Leaves badly injured.
2	In dark room, 7° C., for 4 days.	28	Very few teliospores, on petioles. Infected leaf blades dead.
2	In dark room, 7° C., for 6 days.	—	Leaves killed. No teliospores formed.
8	Checks, in greenhouse at 20° C.	45	A few teliospores on petioles. Leaf blades dead.

In the first set of plants the gradual decrease in metabolism of the host due to the different environments was sufficient to stimulate the formation of the telial stage. That the host was not seriously injured by the treatment was evidenced by the fact that telia were formed on the blades of some of the leaves. In a similar manner the telial stage was produced on the plants which had been in the cold dark room for a period of two days. In this case, however, the blades showed the effects of the darkness and low temperature and had completely withered. The same effects were apparent in the plants which were placed therein for longer periods of time. It is evident that on this host it is difficult to reach the exact condition wherein the uredinial stage will be inhibited and the telial stage produced without killing both the host and the parasite.

As it has been shown by Aeton (1), Boehm (6), and others that roots, stems, and leaves will take up carbon compounds from solution, whole plants of alsike were placed in solutions of sucrose and submitted to varying conditions of light in an attempt to lower the metabolism of the host to a point that would inhibit uredinial production and at the same time not cause the death of the host.

Sixteen plants were inoculated. At the time of uredinial formation, 12 of them were removed from the pots, the roots thoroughly rinsed and cleared of soil particles, and placed in 100 cc. flasks of seven per cent sucrose solution. The remaining four plants were allowed to remain in the pots as checks. Eight of the plants in the sucrose solution were placed in the dark room at a temperature of 19° C. and the remaining four left in the greenhouse with the checks.

The solutions were changed every day and the roots of the plants rinsed in a one per cent solution of Zonite (a commercial preparation of NaOCl held in electrolyzed hypertonic solution), after which they were thoroughly rinsed in distilled water and replaced in the new sucrose solution. In this way contamination was kept very low, and the roots did not seem to be injured by the application of Zonite.

Two days later, four of the plants in the dark room were transferred from the sucrose solution to distilled water for four days, after which they were again placed in the sucrose solution. It was noticed at this time that the petioles of the plants in the dark room contained large numbers of uredinia, while those in the greenhouse in the light had only about one-half as many.

At the end of the tenth day after inoculation, teliospores were observed on the leaf petioles of those plants which had been transferred from the sucrose solution to distilled water and back again. Three days later they were also found on the plants which had been in the dark continuously on the sucrose solution. They were far more numerous on the latter plants, each pustule containing 50 per cent teliospores. On the former the teliospores were few and interspersed with urediniospores in only about 30 per cent of the pustules. Three days later teliospores appeared on the plants in sucrose in the greenhouse, and on the potted plants in the greenhouse on the thirty-eighth day following inoculation.

In practically all cases in this experiment teliospores were formed on the petioles and not on the leaf blades. The leaves of the plants in the dark withered soon after they were placed there, the ones on distilled water dying before the others. It appears that the plants which were on the sugar solution continuously in the dark, although they did not produce the telial stage so soon as those which were transferred to water, absorbed more of the nutrient from the solution and as a result produced more teliospores than

the other plants. The plants on water felt the hunger stimulus first but did not have the food supply to produce the abundant crop as did the others.

Puccinia taraxaci Plowr. on *Taraxacum officinale* Weber. *Taraxacum* rust is similar to *Puccinia suaveolens* in that it produces pycnia accompanied by primary uredinia instead of by aecia. Infections by primary urediniospores produce the regular secondary urediniospores followed by the telial stage. The incubation period of *P. taraxaci* varies from 8 to 12 days; the uredinia first appear on the lower surface, and usually break through the upper epidermis later. The optimum temperature for spore germination appears to be about 18–20° C.

The telial stage of this rust is seldom found in the greenhouse and, according to the experience of the writer, has never been seen there except under experimental conditions as described below. There appears to be great variability in the resistance and susceptibility of host plants to this rust, for plants have been brought into the greenhouse which have never become infected as a result of inoculation, either in pots or petri dishes. Other individuals transferred from the same vicinity in the field showed great susceptibility under both conditions. In the work with this rust, as well as with the others, care has been taken to limit the experimental work to one strain of rust, so that, to the best of the writer's knowledge and belief, discrepancies from this source have been kept out. Any difference in infection ability has been due to the host alone.

Various experiments were tried with light and temperature, similar to those already reported, but in no case under these ordinary methods was the telial stage produced. An infected plant may continue to produce urediniospores until the leaf is entirely covered with pustules. Placing the plants in darkness and under low temperature may cause a temporary lessening of uredinial production, but shortly after the plants are again placed in a favorable situation copious uredinial production is resumed.

Infected plants were placed in the dark at a temperature of 19° C. and also in the dark at 7° C. and left for periods varying from one to seven days, but in no case were teliospores produced. In extreme cases the host was killed without ever showing any signs of the resistant spore. In all, 65 infected plants were used, and not a single teliospore was produced.

Finally an attempt was made to vary the conditions in such a way as slowly to bring the metabolism of the host plant to a condition similar to that existing in nature during the late growing season when the telial stage is produced.

Taraxacum plants were inoculated. Three days after the first appearance of uredinia, plants T10–15 were placed in the cold dark room at 7° C.

for three days. They were then transferred to the greenhouse for seven days and again removed to the cold dark room for six days. Five days after they had been returned to the greenhouse, teliospores appeared in the old uredinial pustules. They were not numerous, averaging about one teliospore to every ten urediniospores. They continued to form for four days, until, under the influence of the greenhouse environment, urediniospores were again produced exclusively. The plants were then transferred to the cold dark room for three days and, three days after their removal to the greenhouse, urediniospore production ceased and teliospores were again formed in the old pustules. This continued for several days to the exclusion of the urediniospores, until finally the infected leaves died, leaving the plant rust-free.

This experiment shows that it is possible to induce telial production even in those plants which do not produce the telial generation readily under the ordinary experimental methods used for some of the other rusts. The principle, however, is the same in this case as in the others.

Puccinia antirrhini Diet & Holw., on *Antirrhinum majus* L. Plants of *Antirrhinum* were raised from seed in the greenhouse and inoculations made in the usual manner. The incubation period of *Puccinia antirrhini* ranges from 7 to 14 days. The optimum temperature for spore germination is about 19–20° C. A temperature of from 25 to 30° C. will gradually free the host of the parasite.

The first evidence of infection on the host plants are light areas on the under surfaces of the leaves. These gradually give way to the uredinia, which seldom extend through to the upper surface. The uredinia are large and circular, and under optimum conditions increase in a radial direction by forming concentric rings of uredinia outside of the original sorus. The uredinial and telial generations are the only ones that have been observed on *Antirrhinum*, and, until Hackey (15) and Mains (23) succeeded in germinating teliospores, they were generally thought to be functionless.

Under ordinary greenhouse conditions, teliospores are seldom produced on actively growing plants. Doran (10) succeeded in producing them on plants which had been gradually deprived of water. This probably accounts for their presence occasionally on old stems of infected plants which have lost their leaves in the greenhouse.

Doran (10) reports finding teliospores on plants outdoors after the low temperature of autumn had set in. The writer has found the telial stage both on the leaves and stems in the field in November, although telia were scarce on the leaves.

The first attempt to inhibit the continued production of urediniospores and so obtain formation of teliospores was made in the same manner as for

the earlier mentioned rusts. Plants were placed in the cold dark room immediately on the appearance of uredinia and allowed to remain at 7° C. for periods of time ranging from 2 to 6 days. On being returned to the greenhouse, the urediniospores continued to form on those leaves which had not been seriously injured by the low temperature. By varying the time left in the dark and allowing the plants partially to recuperate in the light after each exposure to low temperature, teliospores soon made their appearance. The results are given in table 8.

TABLE 8.—*The effect of light and temperature on the time of teliospore formation in Puccinia antirrhini on Antirrhinum majus*

No. plants	Treatment after 10-day incubation period	Formation of teliospores (no. days after inoculation)
4	In dark at 7° C. for 3 days; in light at 20° C. for 10 days; in dark at 7° C. for 3 days	21
5	In dark at 7° C. for 4 days	No teliospores.
5	In dark at 7° C. for 6 days	do
8	Checks, in greenhouse at 20° C.	do

The telial stage appeared only on those plants placed at low temperature in darkness at two different times, with an interval between; this permitted the host to withstand the effects of the unfavorable environment without serious injury. On the plants which were subjected to the same treatment only once, there were no teliospores. It would appear that, similar to the case of the *Taraxacum* rust, it required a more extended and gradual treatment to upset sufficiently the metabolism of both the host and the parasite so that the latter was stimulated to produce teliospores. The tips of the infected leaves on all of the plants were withered, and it was only back of these withered areas that teliospores were produced. They were in most cases interspersed with urediniospores, but on a few of the leaves separate telia were formed near the withered tips. It appeared as if the mycelium of the parasite were spreading from the generally infected area back into the uninfected regions of the leaf and there encountering the conditions which stimulated it to produce teliospores. The region of the leaf surrounding the telia was not withered and did not have the appearance of being seriously injured. The regions around the uredinia were severely injured, and it appeared as if these parts of the leaf had succumbed so rapidly that the fungus did not have time to produce the resistant spores.

In the next attempt to produce teliospores, eight plants were transferred, at the time of urediniospore formation, from pots of earth to flasks of distilled water, the roots only being submerged. The flasks were placed in the

dark room at a temperature of 22° C. for three days. At the end of this period the plants were repotted and returned to the greenhouse. Ten days later teliospores appeared in the same manner as before; most of them were interspersed with urediniospores, but a few were in separate telia. They did not continue to form for any length of time, and the same sori, three days later, disclosed the formation of new urediniospores on the outer edges of the old pustules. Thus it appears that, after the stimulus which had induced the formation of teliospores had been removed, the same mycelium was capable of resuming the production of the uredinial generation.

Uromyces polygoni Fuehl, on *Polygonum aviculare* L. Rusted plants of *Polygonum aviculare* were brought in from the field near Ann Arbor and planted in pots in the greenhouse. Uninfected plants from this general region were also placed in pots and inoculated with material obtained from the infected hosts.

The incubation period of *Uromyces polygoni* varies from 8 to 15 days, the uredinial sori first breaking through the leaves on the lower surface and later extending through to the upper. This rust is exceedingly prolific and will spread over an entire plant in a short time even in the greenhouse. The telial stage forms very readily and is produced upon old leaves of infected plants during the entire growing season.

Infected plants P1-5, on the first appearance of the uredinial stage, were placed in the dark room at 7° C. for four days, afterwards being removed to the greenhouse with the checks. Plants P5-10 were placed in the dark room at 19° C., at the same time for the same length of time.

Four days later teliospores were found on plants P1-5 and plants P5-10. In the case of the former, the production of teliospores was almost 100 per cent on all of the infected leaves. They were produced in the old uredinial pustules and were evident only after the old urediniospores had been scraped away. On plants P5-10 they were not so numerous and were only formed on those leaves which were older and more heavily infected. On the checks teliospores were not yet being produced except on some of the lower, older leaves which had been severely injured by the rust.

In four days urediniospores could be found again on all of the plants, the majority being produced around the outer margins of the old pustules. The plants were again subjected to the same conditions as before, and three days after removal to the greenhouse teliospores were being formed for the second time. They were produced on plants P1-5 until the infected leaves dropped and left the plant rust-free. On plants P5-10 teliospores were produced for several days, then urediniospores were formed again for a short time before the rust finally died. On the check plants, meanwhile,

urediniospores were produced, except on the older infected leaves, for several weeks, until gradually the rust died.

Puccinia triticina Erikss., on *Triticum vulgare* Vill. Plants of the Golden Wave variety of wheat were grown from seed in pots in the greenhouse and infected with material of *Puccinia triticina* received from Dr. E. B. Mains, of the Purdue Agricultural Experiment Station, Lafayette, Indiana. The incubation period varies from 6 to 12 days. The first signs of infection are light-colored areas on the leaves, through which the uredinia soon break. Leaves infected with *P. triticina* soon dry up, and unless they are reinoculated about every 15 days it is impossible to maintain the rust in the greenhouse.

In all of the experiments made with this rust in the greenhouse, the writer was not able to produce a condition of the host whereby the uredinial stage was inhibited and the telial stage stimulated, although such a condition is not to be considered impossible of attainment in greenhouse experimentation.

Rusted plants were subjected to darkness, low temperatures, dessication, etc., but in no case were teliospores produced. They were, however, induced on detached leaves in petri dishes. A description is given later.

Puccinia sorghi Schw., on *Zea mays* L. The effects of light and temperature upon the production of teliospores by *Puccinia sorghi* was demonstrated by several experiments similar to those conducted for rusts so far mentioned.

Mains (21) carried on exhaustive cultural experiments with this organism and gives an account of its reactions, so that further discussion of the preliminary procedure is unnecessary.

Plants were inoculated in the usual manner, and at the time of first uredinial formation were put in darkness and at low temperatures. The results are shown in table 9.

In this situation we find a condition somewhat similar to that present in the wheat. It is difficult to adjust the conditions for control of the metabolic processes in the host in such a manner that the rust will be able to respond without first killing both the host and its parasite. On only one of the four plants placed in the dark room at 19° C. were teliospores produced, and on two of the four placed in the dark room at 7° C. In both of these cases they were very scattered and were interspersed with urediniospores in the center of the uredinia. In the other plants either the infected leaves died, or the region surrounding the sorus appeared to be completely dried out, leaving the rust mycelium isolated from the food in other regions of the leaf. It is interesting to note that in infected leaves exposed to light the areas surrounding the sori retain their green color long

TABLE 9.—*The effect of light and temperature on time of teliospore formation in Puccinia sorghi on Zea mays*

No. plants	Treatment after 6 to 8-day incubation period	Formation of teliospores	
		No. days after inoculation	Remarks
5	In dark room, 19° C., for 4 days	—	Leaves dried up. No teliospores formed.
6	In dark room, 19° C., for 3 days	—	do
4	In dark room, 19° C., for 2 days	20	A few teliospores formed in with the urediniospores on one of the plants.
5	In dark room, 7° C., for 3 days	—	Leaves dried up. No teliospores formed.
4	In dark room, 7° C., for 1 day	17	Teliospores formed on two of the four plants in the old uredinial pustules.
10	Checks, in greenhouse at 20° C.	—	No teliospores formed. The older leaves gradually dried up and left the plant rust-free.

after the other portions have faded, while the reverse is true of plants placed in the dark.

This phenomenon has been observed by Ward (48), Tubeuf (45), and Mains (21) in their experiments with rusts.

Puccinia asparagi DC., on *Asparagus officinale* L. Seeds of asparagus were planted in pots in the greenhouse, and when the plants were about eight inches tall they were inoculated with *Puccinia asparagi* collected in the field near Ann Arbor.

The incubation period of this rust is quite variable, depending largely on the age and condition of the host. Very small plants are not easily infected. In several of the inoculations the time was from 12 to 18 days. In petri-dish culture, to be taken up later, the incubation time ranged from 12 to 20 days.

In all the experiments on the effect of temperature and light the results were negative as far as production of teliospores was concerned. Infected plants were subjected to various conditions but in all cases died before any results could be expected.

Moisture Relations of Teliospore Production

The moisture relation of *Puccinia asparagi* can, according to Smith (40), be considered under two heads; the direct effect of atmospheric moisture on the spores or mycelium of the fungus, and the indirect effect of moisture on the parasite through its effects upon the host. The latter, of course, means soil moisture.

The writer has attempted to verify by experimental methods in the greenhouse the field observations of Smith upon the moisture relations of *P. asparagi*.

Potted plants of asparagus were inoculated with the uredinial stage of the rust and the infected plants apportioned for the experiments as follows:

Two of the plants, A1-2, were placed in the greenhouse where the humidity was relatively high and given only enough water to keep them alive. Two, A3-4, were placed under bell-jars in a well-lighted place with the pots standing in water. The remaining two, A5-6, were placed in the greenhouse by the side of A1-2 and kept well watered.

One week later, on plants A1-2 teliospores appeared in the center of the old uredinial pustules. In another week the entire infected regions of the stalks were filled with teliospores and the fungus had ceased to spread.

On plants A3-4 the rust continued to form urediniospores for two weeks, new sori appearing as concentric circles around the old. The urediniospores were heaped up in the sori with exceedingly long pedicels, and behaved as the rust did when cultivated in petri dishes on detached stalks of asparagus. This is probably due to high humidity, since Hennings (16) observed the same conditions on rusted plants in very humid environments. During the third week after infection teliospores appeared in the center of the first formed uredinia and gradually spread outward but with much less rapidity than in plants A1-2. By the end of the fourth week the pustules were exclusively telial and the infected stalks were killed.

On plants A5-6 teliospores were produced at the end of the second week in the center of the first formed uredinia. The former spread outward in a radial direction preceded by the circles of uredinia. By the end of the third week the sori were completely made up of teliospores and the stalks were dead.

We thus get different results under three sets of different environmental conditions: first, high humidity and high soil moisture; second, moderate humidity and high soil moisture; third, moderate humidity and low soil moisture.

Under the first set of conditions, prolific urediniospore production occurred for nearly three weeks before the telial stage appeared, and even then the new uredinia continued to form for almost two more weeks before teliospores were produced in all of the sori.

In the second case teliospores appeared during the second week, preceded by uredinial production until the end of the third week.

In the last situation the telial stage appeared during the first part of the second week, and before another week had passed the pustules were completely telial in nature and the spread of the fungus had ceased.

It is evident that soil moisture played an important rôle in determining the type of spore produced, for plants A1-2 and A5-6 were exposed to precisely the same humidity, but the amounts of soil moisture differed. That humidity played a part is also evident, as plants A3-4 and A5-6 had practically the same amounts of soil moisture but different humidities.

Four more potted asparagus plants were inoculated and, at the time of uredinial formation, two of them, A12-13, were placed in the south window of the laboratory room, while the other two, A14-15, remained in the greenhouse. Both sets of plants received plenty of soil moisture, practically the only difference in environment being the relative amounts of humidity.

Plants A14-15 developed in the same manner as did A1-2 in the previous experiment, the uredinial sori spreading radially, followed by the production of teliospores in the old pustules. By the end of three weeks spore production had almost ceased, teliospores occupying the whole of the sori.

Plants A12-13 formed teliospores at the end of seven days in the primary and secondary sori previously occupied by urediniospores. No uredinia formed beyond the second ring, and by the end of two weeks the spread of the rust had been checked. The plants were then taken from the laboratory and placed under bell-jars in the greenhouse with plenty of soil moisture in the pots. Six days later new uredinia were being formed exterior to the old sori, followed shortly by teliospores.

The results of the latter experiment added to those of the previous one make it certain that moisture, both in the air and soil, plays an important part in the type of spore produced by the fungus. However, can we say that either has a direct effect upon the fungus parasite? Until we know exactly what influence the shifting of environment had upon both the host plant and the parasite, we are not justified in ascribing to moisture of any kind a direct influence upon the fungus alone. Smith (40) says that when green stalks containing telial material are placed in moist chambers "there immediately breaks out at the outer edge of the infected area a circle of uredosori, with spores capable of immediate germination." This he ascribes to the direct effect of atmospheric moisture upon the rust fungus, without considering that back of the fungus is the living host with its complex of metabolic processes. Did the placing of the stalk in the moist chamber change the metabolic activities of the parasite and leave completely unchanged the cells of the living substratum upon which the fungus was growing? Certainly, from a physiological standpoint, the host in the moist

chamber was a different host than the one on which the teliospores were being produced. To the writer, it appears that, until we can separate the rust parasite from its obligate host and culture it upon an artificial medium, we cannot be justified in assuming that a change in environment of any kind will directly affect the fungus without at the same time having an equal effect upon the cells of the living host. These, in turn, through their change in metabolic activity, will exert a far greater influence upon the obligate parasite than any external factor such as moisture, light, temperature, etc.

Inasmuch as we can safely assert that the living host cells are the sole means of food supply for the rust fungus, and as the increased activity of such cells in a congenial host increases the activity of the parasite, any influence that is favorable for the host cells will be correspondingly favorable for the fungus. Thus when asparagus plants were brought from an environment of low moisture to a more favorable one of high moisture, metabolism increased—more food was made available for the rust—with the result that the uredinial generation was again produced.

In the case of bean rust the moisture relations, while not so marked as in the asparagus, nevertheless were noticeable. On rusted plants slowly deprived of water the telial stage appeared earlier than on plants which were kept well supplied.

Infected bean plants were divided into two lots, one brought into the laboratory at the time of uredinial formation and placed in an east window, the other allowed to remain in the greenhouse. Both were kept well supplied with soil moisture.

Three weeks from the time of inoculation, on the plants in the laboratory, teliospores were forming in the old primary uredinia, while none appeared on those in the greenhouse for another week. In the latter case secondary and tertiary uredinia had been formed, and it was in the tertiary pustules that teliospores appeared. While the light factor may have entered into the experiment to some extent, it was not sufficient to account for the extreme contrast in results. Repeated experiments produced the same results; even when additional light was supplied to the plants in the laboratory by means of electric lamps, the teliospores always appeared from five to seven days sooner than on the plants in the greenhouse.

In the case of snapdragon rust, Doran (10) has already shown the effect of moisture on production of teliospores. In the experiments conducted to repeat those of Doran, the teliospores always made their appearance on the stems and never on the leaves. Infected plants, with uredinial lesions on both the leaves and stems, were gradually deprived of water, and in four weeks after the treatment was begun teliospores were produced on the stems. The leaves meanwhile had dried up, only the uredinial generation

having been produced on them. Those plants which were kept well supplied with water were always killed by the rust before the telial stage was reached.

Petri-Dish Experiments

Eleven rusts, including the nine already mentioned, have been cultured on leaves or portions of leaves floated on water or aqueous solutions in petri dishes. Both urediniospores and teliospores have been produced in eight of these rusts, the type of spore depending in each case on the treatment to which the leaves of each host were subjected. In all the experiments recorded, a five or seven per cent cane sugar solution was used, the former being employed only in the first experiments with bean rust.

Uromyces appendiculatus Fr., on *Phaseolus vulgaris* L. Bean rust is exceedingly easy to culture in dishes and forms its telial stage very readily when placed under conditions that are unfavorable for the host. It is a mistaken notion to think that a weakening of the host predisposes it to the ravages of a rust. Quite the contrary is true. In order to insure a well developed growth of a rust it is quite essential that the host, presuming it to be a susceptible one, be in a healthy vigorous condition (Arthur, 3, and Stakman, 41). If the host is weakened or stunted, the rust either will not develop or will be weakened and produce small pustules and pale spores, and in some cases the uredinial stage will be shortened and the telial stage produced prematurely. A fairly vigorous condition of leaves can be brought about by the use of nutritive sugar solutions; hence it is possible to use the petri dish method for a study of rust reactions.

A few preliminary experiments with bean rust have already been reported upon by the writer (50), and these have shown the ease with which the spore stages can be controlled. During the first experiments it was noted that when bean leaves on distilled water were inoculated, the uredinial pustules were always smaller and paler than when the leaves had been floated on a sugar solution. On leaves on distilled water the pustules seldom increased in number as they did under favorable conditions, as when leaves were on sugar solutions. When teliospores were formed on the leaves on water they usually appeared in the primary pustules, and the rust died in from five to seven days after the first appearance of the uredinial stage. On the other hand, when the leaves were on sugar, secondary and tertiary pustules were usually formed, and when teliospores did make their appearance they were nearly always formed in the two latter sori, seldom in the primary. Quite commonly the rust became so active that the leaf was killed without ever forming teliospores. Frequently the leaf was a mass of uredinia with the spores heaped up to a height of a millimeter or more in the sori. Under such conditions telia were seldom formed.

In all of the dish experiments, the amount of infection played an important part in the appearance or non-appearance of teliospores. A light infection with few sori usually meant a long continuance of the uredinal stage if the leaf was kept on the sugar solution; changing such leaves from sugar to distilled water usually hastened somewhat the appearance of the telial stage. Frequently the reverse was also true. An infected leaf which had been on distilled water was likely to produce the telial stage when suddenly transferred to sugar solution.

Young primordial leaves were taken from healthy bean plants and placed in dishes containing a small amount of distilled water. They were sprayed with a spore suspension and set aside for 48 hours. At the end of this time a seven per cent sucrose solution was poured into one-half of the dishes in sufficient amounts to float the leaves freely. The petioles were clipped and the ends immersed to facilitate the entrance of the nutrient. At the time when uredinal pustules were just appearing on the leaves on sucrose, one-half of them were rinsed thoroughly and placed on distilled water. Likewise one-half of those on distilled water were placed on the sugar solution. The leaves were then set aside for seven days, at the end of which time they were examined.

The following scheme will be followed for designating the various treatments given the leaves in dishes:

A—Leaves which had been continuously on distilled water.

B—Leaves which had been on sucrose continuously.

AA—Leaves which had been transferred from sucrose to distilled water.

BB—Leaves which had been transferred from distilled water to sugar.

A. A fair amount of infection was obtained, but the sori were small and pale in color. The leaves were in a good state of preservation and did not seem to be damaged to any great extent as a result of the rust. In the pustules a few teliospores were interspersed with urediniospores, the latter predominating.

B. Heavy infection was secured, with an abundance of secondary uredinia. The leaves were considerably faded with the exception of the regions immediately surrounding the sori. No teliospores were found.

AA. Infection was almost as heavy as in B, but there were fewer secondary uredinia. Teliospores were abundant in the secondary pustules.

BB. The heaviest infection of all was obtained. There were not so many secondary uredinia, but the primary sori were more numerous and larger than those on the other plants.

One week later a second examination of the dishes was made, with the following results:

A. Growth was very scanty; the sori were very small; and comparatively few secondary pustules had formed. The sori, both primary and secondary, except on those leaves which had unusually heavy infections, contained teliospores almost entirely. The heavily infected leaves had no teliospores; they were in an impoverished condition, and the rust appeared to be famished to a point where it no longer had the ability to form teliospores. Leaves on which teliospores had formed and on which the infection was only moderate, were still green and did not appear to be injured by the parasite.

B. Many of the leaves were yellow and dead. The secondary and tertiary pustules contained an abundance of teliospores. As in A, in cases of very heavy infection, no teliospores were formed, and from the appearance of the leaves it seemed reasonable to suppose that the fungus developed with such intensity and speed that the cells of the host were killed and the fungus was practically isolated from the food supply and perished without the time or ability to form the resistant spore.

AA. There was practically no change in the development of the parasite. The leaves were almost entirely yellowed, and the production of teliospores in the primary and secondary sori had not increased much since the last examination. It would appear that, when water replaced the sugar solution, the fungus gradually stopped growing and producing teliospores.

BB. There was further deterioration of the leaves with not much change in the development of the fungus except that teliospores had formed in the secondary and tertiary sori. The areas immediately surrounding the sori were still green in color but did not appear to be alive. It appeared that this area had been isolated from the surrounding tissue and had dried up without losing its color.

The appearance and condition of the leaves of the bean, both in dishes and when attached to the plant, at the time of teliospore formation, suggested a test for the amount of stored food in the leaf at such a period. As the bean leaves store large amounts of transitory starch and practically no sugar, it seemed reasonable to suppose that, even though the rust existed upon some transitory products of photosynthesis or even upon food of a protein nature, the presence or absence of starch in the leaf would give very good indications of the food situation in the leaf. Accordingly, starch tests were made on leaves from the host plant and in petri dishes at different stages in the development of the rust, *i.e.*, when producing urediniospores and also when forming teliospores. The evident correlation between the amount of starch in the leaf and the type of spore produced at that time seemed to be strong proof that the appearance of teliospores on bean leaves indicated a paucity of carbohydrates in the host cells.

The results of these tests follow :

1. Leaves from plants in the greenhouse tested two days after the first appearance of the uredinial stage had large amounts of starch throughout the leaf. (Leaves for all tests were gathered at four o'clock in the afternoon, so that in these comparative tests translocation had not progressed to any great extent.)

2. In leaves from greenhouse plants tested 10 days after the appearance of the uredinial stage, the starch areas were localized around the uredinia. The areas distant from the fruiting pustules showed little or no starch.

3. Tests made of leaves 28 days after inoculation and bearing teliospores gave almost no test for starch. Even the areas surrounding the pustules gave only a faint starch reaction.

In plants which had been clipped, the reaction for starch was evident for longer periods than on those plants which had retained their trifoliate leaves. In general, the amount of starch in the leaf varied inversely with the number of teliospores.

Tests made on leaves which had been floated on sucrose solutions and distilled water in petri dishes showed the same variation :

1. Lightly infected leaves on distilled water showed a fair starch reaction at the time of uredinial formation.

2. Leaves on distilled water with heavy infections showed little or no starch at the time of uredinial formation. Such leaves usually had no teliospores.

3. Infected leaves on seven per cent sucrose solution gave an extremely strong starch reaction at the time of uredinial formation, the starch being denser along the cut edges of the leaves and along the larger veins.

4. Infected leaves on sugar at the time of telial formation had little or no starch. In cases of heavy infection where no teliospores were formed, there was practically no starch.

Attempts were made to show the effects of low temperatures upon the formation of teliospores similar to experiments made upon the plants in the greenhouse, but the results were negative.

It was found, on bringing the leaves from low to high temperatures, that rapid decomposition set in, which made it impossible to work with them. Another reason for the inability to perform temperature experiments with detached leaves is the fact that at low temperatures very little starch is formed in the leaves and the development of the rust is at a stand-still. The reaction of leaves and their storage of starch at low temperatures has been investigated by Czapek (9), Lidforss (19), and Tolenaar (44), who found that starch storage took place at low temperatures to a much less degree than at high.

Uromyces trifolii Lev., on *Trifolium hybridum* L. Infection of alsike leaves with *Uromyces trifolii* was easily obtained either on distilled water or the 7 per cent sucrose solution. In the former case the pustules were smaller and paler in color than those formed on the leaves on sucrose. That alsike leaves take up sugar from solution and store it as starch was made evident by iodine tests similar to those made on the bean. Figure 2 shows the result of one such test.

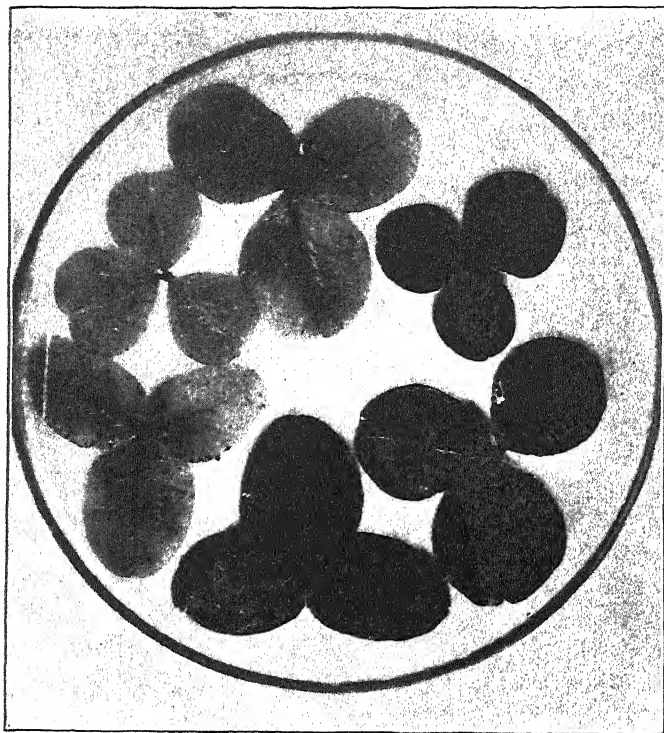


FIG. 2. Starch tests made on leaves of alsike bearing the uredinial stage of the rust. The three leaves on the left were floated on distilled water while the three on the right were floated on a seven per cent sucrose solution.

Preliminary experiments were conducted with alsike leaves by placing some of the dishes containing the leaves in the dark and others in the light during incubation. It was found that with leaves floated on sugar solutions good infections could be obtained if they were kept in total darkness during the whole period of incubation. In the case of the leaves on distilled water, however, it was necessary to place them in a well-lighted position to insure good infection. In some few cases scattered pustules were formed on those

leaves which had remained in the dark during incubation, but this can be attributed to the fact that there was a sufficient amount of stored food already in the leaves to insure at least a scanty infection. Unlike the situation found in the bean leaves, teliospores were found in only two cases out of hundreds of trials with leaves on distilled water. In these exceptions, the number of sori was very few on the leaf, in one instance numbering 20, in the other, 28. Normally, by the spray method of inoculation, 2 to 500 pustules are usually formed on a single leaf; hence it appears to be a case of the fungus famishing before being able to produce the telial stage.

To induce the formation of teliospores, various methods were tried. It has been shown in the experiments with the various host-plants in the greenhouse that exhaustion of the food supply in the host, either through lack of light or moisture, or low temperatures, seemed to inhibit further production of urediniospores with the resulting formation of the telial stage. Methods were therefore used in the petri-dish cultures to control the food supply of the host. It was found that teliospores could be induced in the leaves of alsike by three methods:

1. Starvation of the host, with the later addition of a rich food supply.
2. Sudden change of the host from a well-fed condition to a stage of starvation.
3. Continued supply of food, with the gradual dying of the host cells and lowering of metabolism.

These objectives were attained by the addition and subtraction of the nutrient solutions. The efficiency of the method is, of course, much lower in this case than in working with fungi which can be cultured directly on artificial culture media, because we have the living host, with all of its complexities of metabolism and growth, interpolated between the nutrient solution and the fungus. Nevertheless, sufficient data were secured to justify the conclusions. The attempt was also made to show the effects of low temperature on the leaf and rust metabolism as in the case of the bean leaves, but the same difficulties were encountered and that phase of the experimentation was suspended.

Table 10 shows the results of infection experiments conducted with detached leaves. The results show a relationship between the presence of carbohydrates in the leaves and successful infection.

Experiments were next conducted to show the effects of changing the nutrient solutions on the infected leaves. Alsike leaves in 32 dishes were inoculated, and at the end of 48 hours sucrose was added to 16 of them, the other 16 receiving distilled water.

Eight days after inoculation, uredinia appeared on the leaves on the sucrose solution and one day later on the leaves on distilled water. At this time seven dishes of leaves were changed from distilled water to sucrose, seven were allowed to remain on the water, six were changed from sucrose

TABLE 10.—*The effect of light and medium on development of Uromyces trifolii on detached leaves of Trifolium hybridum*

No. dishes containing detached leaves	Medium	Incubation period in days	Light condition during incubation	Infection
12	Distilled H ₂ O	7	Light	Sori small and pale in color.
8	do	10	Dark	Only two out of eight leaves infected.
8	7% sucrose solution	6-7	Light	Good infection; 200-500 pustules per leaf.
8	do	7	Dark	Fair infection, large pustules; 50-100 pustules per leaf.

to water, and ten remained on the sucrose. The solutions were changed every other day to prevent contamination.

Twenty days after inoculation the leaves were examined. The results were as follows:

1. The leaves which had been on distilled water continuously were all lightly infected, and on one leaf that had a lighter infection than the others there were teliospores formed in the old uredinial pustules and interspersed with the urediniospores. The other leaves were in a fair state of preservation, but the rust was not developing any further.

2. On the twenty-second day after inoculation teliospores were found in five of the seven dishes of leaves that had been transferred from water to sucrose. The teliospores were quite numerous and in some cases were formed in separate telia; the majority, however, were formed in the old uredinial pustules.

3. Examination of those leaves which had been on the sucrose throughout the experiment revealed only a very few teliospores. Uredinia were formed in very large amounts and the leaves had the appearance of being almost destroyed. As was found to be the case with most of the rusts, a continued supply of nourishment almost universally caused the fungus to produce the uredinial stage until finally the host was exhausted so suddenly that there was no time for the formation of teliospores.

4. The rust on leaves that had been transferred from sucrose to water had grown practically not at all after the sucrose was removed. For several days after the nutrient was taken away a few urediniospores had been formed, but not many. No teliospores were formed, and the leaves soon disintegrated.

As no teliospores formed when the leaves were transferred from sucrose to water at the time of uredinial formation, it was thought advisable to transfer them before the fungus could progress that far in its development and before it could appropriate too much of the stored food in the leaf for the production of urediniospores. Accordingly, leaves in 16 dishes were inoculated in the regular manner and set aside for only 48 hours. At the end of this time, the leaves in eight of the dishes were supplied with 7 per cent sucrose solution, the leaves in the other eight remaining on distilled water. Six days after inoculation, leaves from four of the eight dishes were rinsed and transferred from sugar to distilled water.

From four to six days later, uredinia appeared on practically all of the leaves in the 16 dishes. Twenty days after inoculation teliospores appeared on the leaves which had been transferred from sugar to water. The other leaves showed about the same results as those outlined in the preceding experiment. The fact that teliospores formed on leaves which had been transferred from sugar to water before uredinia had formed made it appear that the sudden removal of food at such a stage of development inhibited production of urediniospores and stimulated the immediate formation of teliospores.

Puccinia suaveolens Rostr., on *Cirsium arvense* (L.) Scop. *Puccinia suaveolens* was not at first readily cultured on leaves of *Cirsium arvense* in dishes due to their rapid discoloration and decomposition. In the early part of the work it seemed almost impossible to obtain infection. Finally it was found that by selecting the younger and more succulent leaves for inoculation a large percentage of them became infected.

Experiments were conducted to determine the ability of the *Cirsium* leaf to take up sugar and store it as starch in the cells, and it was found that large amounts were stored after four or five days on the 7 per cent sucrose solution.

Infections of rust on leaves floated on distilled water, if not too heavy, produced teliospores a few days after the first appearance of the uredinial stage. During winter days when the light intensity was low it was not infrequent to find that the telial stage was the first and only stage of the rust to appear on the leaves on water.

That the presence or absence of food in the leaf was a deciding factor in teliospore production is shown by the results of the following experiment. Leaves in 28 dishes were inoculated, and 48 hours after inoculation sucrose was added to 14 of them, the leaves in the remaining 14 being left on the distilled water. From five to six days later uredinia appeared on practically all of the leaves, and at this time one-half of the leaves on sucrose were rinsed and placed on distilled water, and one-half of those on water were placed on sucrose.

Fifteen days after inoculation, teliospores were found in large numbers in the old uredinial pustules of those leaves which had been continuously on distilled water: also they were being formed on those leaves which had been transferred from the sugar to the water. In the latter case they were not at all numerous and the leaves were severely injured. Various results were obtained with the leaves which had been continuously on the sugar solution. Some of the leaves had teliospores and some died after producing the uredinial stage. In all cases where the leaves remained in good condition and the infection was not heavy, teliospores were formed.

On leaves which had been originally on water and were transferred to the sugar solution, most interesting phenomena were observed. The same sori which had originally produced urediniospores and later formed teliospores again formed urediniospores when the effect of the transfer to the sugar solution was felt. The later-formed urediniospores were not very numerous but sufficient to show that by the addition of the sucrose a new food supply had been provided for uredinial production. Thus we find the following spore forms being formed successively: urediniospores, as a result of the first infection; teliospores, as a result of the distilled water for the first eight days: and lastly, the second production of urediniospores as soon as the sugar solution had had an effect. It is interesting to note that some of the leaves which were producing urediniospores for the second time were completely brown: scarcely a trace of the original green color was to be seen.

As has already been explained, the time of year with its corresponding light intensity was a deciding factor with those leaves which were put on distilled water only, and which supplied to the rust only that food which was being formed in the leaves through photosynthesis carried on while the leaves were in the dishes.

Puccinia antirrhini Diet and Holw., on *Antirrhinum majus* L. Attempts were made to culture *Puccinia antirrhini* on *Antirrhinum* leaves on solutions, but infections were so scanty that reliable conclusions could not be drawn. The leaves themselves remained in good condition in the dishes for five or six weeks either on nutrient solutions or on distilled water. Occasionally adventitious rootlets were put out from the petiole and would grow until they attained lengths of ten centimeters or more. In one instance a rooting leaf was placed in a flask with Shive's solution and remained alive for 60 days; in attempting to induce it to grow in a pot of earth, decomposition set in and it was destroyed.

The leaves were finally infected on the lower surface, as has been explained elsewhere in the paper, and from then on good infections were always obtained.

As has been noted in the work with *Antirrhinum* rust in the greenhouse, the telial stage is rare under greenhouse conditions. Likewise in the dish cultures, the uredinal stage may continue to form for long periods of time on sugar solutions and the telial stage may never appear. On infected leaves on distilled water the pustules may dry up before more than just the primary uredinal pustules can be formed.

Early in the experimental work before a successful method of inoculating the leaves had been discovered, a few experiments were conducted with cuttings in solutions. Ten cuttings were inoculated, five of which were placed with the stems in flasks of distilled water and five in a 7 per cent solution of sucrose. They were kept during the whole of the experiments under bell jars to prevent excessive transpiration. Six days after inoculation, when the first signs of uredinal production became visible but before the pustules had broken through the epidermis of the leaves, three of the cuttings in sucrose were transferred to distilled water. The others were allowed to remain in the same solutions.

On the sixteenth day after inoculation, teliospores were found on the leaves of the cuttings which had been transferred from the sucrose to the distilled water. They were not numerous and were interspersed with urediniospores in the original uredinia. There were no teliospores on the leaves of the other cuttings. The pustules on the leaves of the cuttings in the distilled water were dried up as a result of rapid starvation or desiccation. On the cuttings in the sugar solution secondary uredinia were produced in abundance, but due to contamination it was impossible to determine whether teliospores would eventually have formed or not. While these results are not in themselves especially conclusive, when taken in conjunction with the results obtained later with detached leaves on solutions they show the relationship between the available food in the host and the production of teliospores.

In experiments with the detached leaves alone, it was found that leaves which were on sugar solutions continuously, if the amount of infection was not too heavy, usually produced the greatest abundance of teliospores. Rust on leaves which were transferred from sugar to distilled water, if the transfer was made before the fungus had advanced too far in its development, generally produced the telial stage.

Table 11 gives the results of some of the experiments conducted with *C. antirrhini* on detached leaves of *Antirrhinum*.

In experiment A, leaves which were on distilled water continuously did not form teliospores because, as has been found to be the case with all of the rusts, when the infection was heavy the rust famished so quickly that the production of teliospores was impossible. In Experiment B it is seen that when the infection was light teliospores were formed even though the

leaves were on distilled water all of the time. In Experiment C the same results were obtained as in Experiment A under the same conditions. The infection in the case of the leaves on distilled water was too heavy to allow teliospore formation. The other results were similar to those obtained with other rusts with the exception that when *Antirrhinum* leaves were transferred from distilled water to sugar solutions the leaves failed to take up the sugar. As a result, in Experiment A, where the leaves were transferred from water to sugar, the rust failed to respond to the change in environment and gradually died out without producing any more spores.

TABLE 11.—*The effect of different media on teliospore formation in Puccinia antirrhini on Antirrhinum majus*

No. dishes used	First nutrient	No. days on first nutrient	Second nutrient	Incubation time in days	Formation of teliospores (no. days after inoculation)
Experiment (A)					
6	Distilled H ₂ O	10	7% sucrose	9-10	None
5	do	10	Distilled H ₂ O	9-10	do
7	7% sucrose	10	do	9-10	19
7	do	10	7% sucrose	9-10	25
Experiment (B)					
8	Distilled H ₂ O	17	Distilled H ₂ O	8	17 ^a
8	7% sucrose	21	7% sucrose	8	21
Experiment (C)					
12	Distilled H ₂ O	25	Distilled H ₂ O	10	None
14	7% sucrose	25	7% sucrose	8	25

^a Infection very light; average no. of pustules per leaf about 20.

Puccinia sorghi Schw., on *Zea mays* L. *Puccinia sorghi* was readily cultured on leaves in petri dishes and, as Mains (21) reported, good infection could be obtained in the dark when the leaves were floated on sugar solutions.

Teliospores were induced in this rust by the same general means as in the other rusts already discussed. If sucrose was added at a time when the rust was beginning to feel the effects of the diminishing food supply, teliospores were formed. They were found on leaves which were on sucrose continuously, and also on leaves which were transferred from sucrose to distilled water before the uredinial pustules were fully formed. They were never produced on leaves floated on distilled water only. It was found that, if leaves were transferred from sucrose to distilled water after the uredinia were formed, teliospores were never formed; if, however, the

transfer was made three or four days after inoculation, the telial stage always appeared. The teliospores were in all cases interspersed at first with the urediniospores until finally there were only teliospores in the pustules.

Puccinia triticina Eriksson, on *Triticum vulgare* Vill. The experiments with *Puccinia triticina* were but repetitions of the ones with *P. sorghi*, for these two rusts and hosts seemed to behave similarly. That there is a difference, however, is evidenced by the fact that teliospores of *P. triticina* are seldom, if ever, seen on young plants of wheat in the greenhouse. Mains (22) says of this rust: "*Puccinia triticina* upon wheat seedlings has never been seen to produce telia under such conditions even on old dying leaves, but does produce some telia on old leaves when the wheat plants approach maturity." Teliospores were produced on a few young corn plants in the greenhouse but they were never induced on wheat.

On the leaves in dishes the writer has been able to produce teliospores with the same ease as on corn leaves.

To avoid the contamination that is so likely when culturing wheat leaves, leaves which were already infected on the plant were used. They were taken from the infected wheat plants before the uredinia had broken through and placed upon distilled water in the dishes. After three days, one-half of the leaves were placed upon the 7 per cent sucrose solution, the other half remaining upon distilled water.

Five days later an abundance of teliospores was found upon the leaves which had been transferred to sugar. They were formed in linear sori in most cases but some were interspersed with urediniospores in the old uredinia. There were many abnormal spores: some thick-walled, one-celled with spines; others two-celled with spines; and some like the typical two-celled, smooth-walled teliospore.

As was mentioned above, wheat and corn leaves cultured in dishes were more liable to contamination than any of the leaves belonging to the dicotyledonous group. Dicotyledonous leaves have been kept in dishes for several weeks with few signs of contamination, but corn and wheat leaves usually were covered in a few days with yeasts and other contaminating fungi.

This can probably be explained by the fact that in the monocotyledons there is usually an abundance of glucose and other monosaccharides stored within the leaf while in the dicotyledons there is little sugar stored as compared with the large amounts of starch (Parkin, 32). As the monosaccharides are easily attacked by the average saprophytic fungus, it is natural to expect such leaves to be contaminated sooner than those containing insoluble starches. In placing the leaves in dishes it is sometimes neces-

sary to cut the edges, and it is at these points that there is probably a diffusion outward of the stored sugars. These regions afford a favorable place of attack for the saprophytic forms.

Puccinia taraxaci Plowr., on *Taraxacum officinale* Weber. *Taraxacum* rust is easy to culture on leaves in dishes, but the formation of the spore type desired is very difficult to control. Uredinial formation continues sometimes up to the point where the whole leaf is destroyed. In many cultures upon sucrose solutions urediniospores continued to form for seven weeks, and even up to the point where only a small fragment of the leaf remained in the dish, new urediniospores were being formed.

The rust was grown upon both sugar solution and distilled water and the usual transfers made from one to the other, but with no results other than the continued production of urediniospores or the death of the leaves. Finally it was found that by transferring infected leaves from the sugar solution to distilled water and then back again, teliospores were produced.

The leaves were first inoculated in the usual manner on distilled water and, after two days to allow for spore germination, were transferred to sucrose solution. Here they remained for 16 days, in the meantime producing secondary rings of uredinia. They were then rinsed thoroughly and placed back again upon distilled water for five days, and then finally replaced upon the sucrose solution. Four days after the last transfer, examination revealed teliospores interspersed with urediniospores in some of the secondary sori. They continued to form for several days, but later urediniospores were formed instead. These continued to form for several days until the leaves became so contaminated that they were discarded. New sets of leaves were tried, and in each case teliospores made their appearance in practically the same manner and under the same type of manipulation.

The results of these experiments, although somewhat different from those for the other rusts, show the same relationship between food condition and production of teliospores. The host requires a further state of exhaustion before the telial stage is produced. That this is true can be determined from field observation on the nature of the host and the rust. A study of this rust over a period of three years in the field has failed to reveal a single case where the telial stage has been present except in the late summer or fall. This shows that the metabolism of the host and the fungus are so closely adjusted that the telial stage is produced under only the most well adjusted conditions. Magnus (20) has noted this situation in the *Taraxacum* rust and has explained it as being due only to the fact that the host is so hardy that its metabolism is not readily upset. Quite in contrast with this rust is that upon *Cirsium* where the least unfavorable condi-

tions for the host immediately throw the spore production of the parasite from the uredinial to the telial stage. A study of the life history and habits of these two hosts, however, seems to the writer to explain fully the differences in sensitiveness of the two. Both conditions are the result of a long period of evolutionary adaptation of each rust to its particular host. The same relationship exists in each case, and it is only when the experimenter has succeeded in finding the exact condition that upsets the perfect balance between host and parasite that the rust is stimulated to produce the resistant spore. The same principle holds true in each case, the only difference being the ability to simulate the proper conditions.

Puccinia orbicula Pk. & Clinton, on *Prenanthes alba* L. Perhaps the most striking demonstration of the effects of food conditions on the type of spore produced by the rust parasite was made with *Puccinia orbicula*. On the very young plants urediniospores and teliospores accompany the aecia quite profusely if the sorus occurs adjacent to the midrib or the larger veins. Generally upon the stems there is an almost pure teliospore production. The larger and more developed leaves contain a greater percentage of urediniospores than the younger. To all outward appearances this rust is somewhat similar to the *Podophyllum* rust in the distribution of the teliospores. Just as Whetzel, Jackson, and Mains (51) termed the latter "a plastic rust," so it appears that the *Prenanthes* rust might be regarded as quite plastic.

Healthy leaves of several ages were brought in from the field and infected with material obtained from rusted plants. Dishes P1-4 contained leaves which were about two-thirds mature floated on sucrose, the margins being cut to facilitate the absorption of the nutrient. Dishes P5-8 contained large, more matured leaves, but with only the petioles immersed in the sugar solution. Dishes P9-12 contained very young leaves on distilled water. Dishes P15-18 contained very young leaves with their petioles immersed in sucrose solution, and dishes P15-18 large leaves floated on distilled water.

Four days after inoculation, the large leaves with the cut margins had become thoroughly reddened due to the formation of anthocyanin, the leaves with only the petioles immersed becoming progressively red as the solution traveled up the veins.

On June 10, or 11 days after inoculation, urediniospores appeared on the leaves in dishes P1-5. The sori were very large and quite numerous. The same day they appeared on leaves P5-8, but were not so large nor so numerous. On the next day the uredinia appeared on the remaining leaves; in the case of P9-12 they were small, few in number, and contained about 90 per cent teliospores; P13-15 showed fair infection with many teliospores



FIG 3. Rusty leaf of *Prenanthes alba* which had been transferred from distilled water to sucrose solution after having been on the distilled water for four weeks. New uredinia are seen breaking through the epidermis around the old telia

accompanying the urediniospores. Leaves P15-18 had a few scattered sori, but in all cases they proved to be almost wholly filled with teliospores; only a very few urediniospores had formed.

Three days later, all of the leaves were producing teliospores. On the leaves in dishes P1-4 urediniospores were produced for the longest period; on the others teliospores were found in the same ratio as given above. The experiment was abandoned with the exception of dishes P15-18. The plants in these dishes were allowed to remain on distilled water for four weeks, and then placed on sugar solution. Four days after the transfer, examination revealed the surprising fact that the leaves had become decidedly red in color and new uredinial pustules were breaking through the epidermis (Fig. 3). The pustules could only have been the result of mycelia that had been dormant in the leaf for some time; and the sudden addition of the nutrient had renewed growth so that uredinia were produced. It must be remembered that these were the same leaves that four weeks earlier had shown only telial formation on the distilled water.

The dishes had remained closed for the preceding few weeks and there was little chance that the infections were the result of urediniospores from the outside. To the writer the logical conclusion appears to be that the type of spore which had been first produced and that which was later produced was determined wholly by the presence or absence of the carbohydrate within the cells of the leaf.

DISCUSSION

General Principles

It is evident that there exists between the host and its rust parasite a very close and intimate relationship, with the life processes of the two so closely attuned that a slight disturbance in the metabolism of the former is sufficient to disturb the latter and cause it to pass from a stage of active fructification, as evidenced by the uredinial generation, to one of quiescence, the telial. In extreme cases the uredinial stage may be entirely suppressed and then only the telial stage appears.

That the host does possess a very direct influence upon the fungus has been shown by Arthur (3), who points out very clearly that, as a rule, the vigor of the parasite is directly proportional to the vigor of the host; and that to be successful in culturing the rust fungus on its host it is necessary to use strong, rapidly growing plants. Stakman (41) believes that whatever favors the normal development of the host is ordinarily conducive to the vigorous development of the parasite. Ward (49) found that a starved host meant necessarily a starved rust, while Stakman and Levine (43) have shown that environmental conditions unfavorable to the host were also unfavorable to the rust. That the virulence of the disease may be directly

correlated with the "vegetative vigor" of the host has been pointed out by Raines (33). Sheldon (38) concluded that the lowered vitality of the host does not favor infection and anything affecting the growth of the host also affects in like manner the rust.

With the opinions and results of these investigators in mind, let us consider for a moment the source and nature of the food of the fungus, and see whether the above conclusions and those to follow do not show definitely that the rust fungus is dependent upon the photosynthetic activity of the host; and that any factor, or set of factors, such as temperature, light, moisture, etc., or a complex of these factors, such as climate, will so influence the metabolic condition of the host that it will be reflected in the type of spore produced by the rust. Until we can definitely separate the rust fungus from its obligate association with the host, and study the direct relationship of the fungus to such environmental factors as light, temperature, etc., while culturing it under strictly saprophytic conditions, we are not justified in assigning specific external influences to it. We are compelled to consider the rust fungus in terms of its most influential environment, viz., the host.

To determine the dependence of the rust fungus upon the photosynthetic activity of wheat seedlings inoculated with *Puccinia graminis*, experiments were made by Stakman and Levine (43). They concluded that "Inasmuch as the photosynthetic activities of the host plant are affected by the light intensity, in so much does the structure and function of the rust depend on the same factor."

That the rust is dependent upon intermediate products of photosynthesis was advocated by Fromme (12), while Mains (21) suggested that it is probably dependent upon some transitory or nascent organic products related to the carbohydrates.

Similar evidence of the dependence of the rust upon the photosynthetic activity of the host was obtained by the writer (50) in the case of bean rust, *Uromyces appendiculatus*, and in the present experimental work in ten additional species of rusts. It has been found in all the experiments conducted that once the parasite has gained entrance to a susceptible and congenial host, those conditions which guarantee an optimum supply of carbohydrate food for the host likewise insure a vigorous development of the fungus, as evidenced by a copious production of urediniospores. If, however, through the influence of internal or external factors, or a complex of such factors, the host is no longer able to maintain this condition of vegetative vigor, coincident with its ability to increase and maintain the carbohydrate content of its cells, then the fungus will, if still able to function, react to such conditions, and produce the resistant or telial stage.

Iwanoff (18), who attempted experimentally to prove the direct effect of climate upon certain rusts, illustrated this point in an irrefutable manner. He found that when he transported infected plants from regions of low altitudes to alpine stations he obtained a suppression of the uredinal stage with the hastening of the telial generation. He obtained the same results when he placed infected plants in an ice-chest in the regions of low altitude. The writer also produced the telial stage in seven species of rusts by the latter method. In the case of bean rust, when infected plants bearing uredinia were placed in a cold chamber at a temperature of 7° C. for two days, the telial stage appeared 13 days earlier than on the checks which remained in the greenhouse. Similar results were obtained in the other six species.

Morganthaler (28) transported plants of *Veratrum album* L., infected with *Uromyces veratri* (DC.) Schr., to alpine regions and found that the telial stage was produced sooner than in those plants which remained at low altitude.

Now, do any of these results justify the conclusion that the climate as such or the temperature in itself was directly influential in producing the telial stage? Is it logical to assume that the bean host represents the same set of food conditions at 7° C. as it did at 20° C? In a similar manner, can Iwanoff say that the same rate of metabolism was going on in his host plants at the plains station at an average temperature of 20° C. and an altitude of 520 meters, as was present at the alpine station Faulhorn at an altitude of 2684 meters and a temperature ranging from 2 to 28° C.? Morganthaler took account of these questions and concluded from his experiments that the climate was not directly responsible, but that the change in the altitudinal position of the host plants affected their nutrition, probably retarding it, and in this way induced the formation of the teliospores. He furthermore demonstrated that, by cutting the veins of leaves and by detaching or singeing leaves of *Veratrum album* infected with *Uromyces veratri*, he could induce the telial stage of the rust. He thus concludes that he is not far wrong in assuming that the principal cause for teliospore formation is the diminishing of food; that teliospores assume the role of resistant spores in the life history of the rusts and that the taking away of food stimulates their appearance.

Gassner (14) likewise was unwilling to ascribe to climatic influence the simple direct action that Iwanoff suggests. In his opinion the climate always acts upon the rust in a roundabout way through the host. He showed through extended observations and experiments that, under normal conditions of growth, the time of appearance of the telial stage of grain rusts has a certain relation with the stage of development of the host plant.

This usually occurs at the time of, or just preceding, the heading out of the grains, when there is a general mobilization of food within the plant for seed production. This stage he called the point of exhaustion of the host and assumed that at this stage the diminution of the food caused the fungus to produce the telial stage. He also showed that different hosts reacted differently in that stage of exhaustion. This was illustrated by *Puccinia triticina* and *P. graminis*; he succeeded in obtaining the uredinal stage of the latter upon hosts which were already bearing the telial stage of the former. It appears, however, that Gassner, although he was close to the fundamental issue of the matter, associated too closely the developmental stage of the host with telial production. Practically all his experimental work was done in the field, where environmental conditions other than purely climatic were not operative. In only two or three instances does he mention any attempt to use experimental means to hasten or retard telial formation. In the one case he cut notches in the leaves of the rusted hosts but with no results, the leaves dying soon after. The fungus on starved plants grown on quartz sand failed to produce the telial stage. By decreasing the light he did succeed in inducing the formation of teliospores on infected oat plants sooner than on the checks.

It would seem that even though, in the grain rusts, the telial stage usually accompanies a certain stage of development of the host, as Gassner has shown, it is only that the food situation at that time is in a state of mobilization for seed production and as a result the fungus is cut off from its food supply. The condition, under which the fungus suffers from nourishment inadequate for urediniospore production, may be brought about in a number of ways, so that the particular phase of the story brought out by Gassner is but one angle of the situation. The writer produced these same conditions by subjecting the infected hosts, without regard to age or stage of development, to low temperatures, lack of light, insufficient moisture, and by floating detached leaves upon various nutrient solutions. It is quite natural that under field conditions the telia would appear normally at a certain stage of development of the host; but the same host, under varying conditions of environment, may be so affected as to produce the telial stage at any or at all periods in its life history.

This is illustrated in the case of *Puccinia sorghi*. Gassner (14) found that on plants of *Zea mays* the telial stage appeared at about the time of flowering, *i.e.*, when the plants were about three or four months old. In the present paper it is reported that teliospores were produced on corn plants which were only 30 days old, and which were exposed to low temperatures for only one day. In this case it is evident that the stage of development of the host was not important.

The experiments described in the present paper are but a few among many that could be conducted to illustrate this fact. To bring about the proper balance of food supply between the fungus and the host to produce the telial stage is a problem that depends entirely on the experimental technique and that only continued trials will solve. This is seen in the cases of the different hosts used in the temperature experiments. In the behavior of the *Cirsium* and *Taraxacum* rusts the same principle is involved, with the same outcome in both cases; but to bring about the same results in the two hosts a different procedure is required. As mentioned previously, when *Cirsium* leaves were placed in the cold room for five days the telial stage was produced eight days sooner than on the checks. In the case of *Taraxacum*, placing the infected plant under the same conditions for seven days failed even to stop uredinial production. Three days in a similar environment completely killed the corn plant. Thus, it is evident that the structure and habit of the host plant must be taken into consideration as well as the environment to which it is accustomed. Having discovered the conditions under which the metabolism of the host can be regulated, it is safe to assume that the rust will correspondingly reflect the varied conditions in the type of spore which it will produce.

In attempting to interpret the results of the temperature experiments with such seemingly divergent rusts as those on *Taraxacum* and *Cirsium*, we must consider the nature of the two hosts as found under field conditions. *Cirsium* grows very rapidly: its life processes are carried through in exceedingly rapid succession. In three weeks a plant two or three inches in height may become several feet tall and have fully developed flowers. Under such conditions it is evident that there must be speedy assimilation and translocation of food. The rate of metabolism of the leaves becomes progressively slower as the tissues rapidly mature, and to a parasite inhabiting the leaves of such a plant the food becomes quickly unavailable. What is the result? As one would expect, either the fungus becomes adapted to such conditions and is capable of quickly protecting itself or else it is exterminated. Such protection comes through the formation of teliospores. These are produced at the first sign of waning metabolism, and by the time the food is completely diminished the telial stage is formed. Likewise, under experimental conditions, when the infected host is subjected to any abnormal environment retarding its metabolism, the rust will quickly respond by producing teliospores. Hence, when *Cirsium* leaves were placed under the influence of low temperature, absence of light, or when the detached leaves were transferred from sugar solution to distilled water, the results were identical with those obtained when the host approaches maturity in the field. Similarly, the telial generation is produced when leaves are placed continuously upon

distilled water if, on such leaves, there is a light infection. Ordinarily, on heavily infected leaves, which obtained no nutrient other than that which was synthesized within the dishes, no teliospores were produced. In other words, the fungus starved before telia could be formed. A similar case has been referred to by Gassner in answering the objections of Lagerheim, who maintained that the telial stage does not appear with the exhaustion of the host plant, as he had observed wilted plants of *Vicia faba* L. infected with *Uromyces fabae* de Bary in Quito, Ecuador, on which only urediniospores were visible. Gassner points out that this is probably due to the fact that the plants had wilted suddenly and were not able to reach maturity. He uses this example to point out that wilting and exhaustion are not identical. However, if Gassner had changed experimentally the environmental conditions of this same host he probably would have found that even as wilting and exhaustion are not identical conditions, neither is exhaustion of the host associated necessarily with its later or maturing stage of development.

In the case of the *Taraxacum* rust, where we find apparent differences in reaction, closer analysis reveals the same underlying influence of nutrition. Under the ordinary methods of greenhouse culture no teliospores were ever produced on the *Taraxacum* plant, for the uredinial stage persisted until the death of the infected host parts. But, when the host was subjected to successive exposures of low temperature, the telial generation made its appearance, as demonstrated in plants T10-15.

Taraxacum grows rather slowly in Michigan. The leaves are numerous and grouped closely upon a central axis, with no necessity for rapid assimilation or translocation of elaborated food over long distances. The plant may be found growing more or less actively in almost any month of the year. One would expect the food situation in such a plant to be stable, with no such rapid fluctuations as occur in *Cirsium*. In such a host the sudden diminution of available food, at least during the growing season, is not felt by the rust parasite. It is only with the advent of short days and low temperatures, when there is a gradual withdrawal of food from the leaves of the host, that the fungus is stimulated to produce the telial stage. The life processes of the host and fungus are so in accord that the latter is a direct result of the former. Any treatment that will bring about this unfavorable condition of food in the host will cause the fungus to produce the telial stage. Such a condition was produced when *Taraxacum* was subjected to alternate periods of low temperature and absence of light. Afterwards, when the host was returned to a favorable environment, the rust resumed its formation of the uredinial generation.

The same correlation between the type of host employed and the method

of manipulation required to produce the telial stage of the rust inhabiting such a host is seen in petri-dish culture.

In the case of *Cirsium*, teliospores appeared on the leaves in the dishes under several sets of conditions. If the leaves floated on distilled water were not too heavily infected, the telial stage appeared soon after the first appearance of the uredinia. Likewise, if the leaves were transferred from the 7 per cent sugar solution at the time of uredinial production to distilled water, the telial stage appeared. On the leaves which were continuously on sugar, providing the infection was not too heavy, teliospores formed also, but only after a longer period of time. It is possible that if the leaves could be so kept that they would continue to absorb the carbohydrate gradually, the uredinial stage would persist for much longer periods of time. In spite of all precautions, it is probable that the outer layers of cells adjacent to the cut surfaces die either as a result of bacterial action or autolysis, and further absorption ceases. In such cases, of course, telial formation occurs.

Another explanation which might be presented to explain this cessation of uredinial development on the leaves is that the rusts may not exist upon a purely elaborated carbohydrate diet but require some products related to the proteins. In such an event, when the leaf is left continuously upon the sugar solutions its cells would soon be depleted of such mineral nutrients as nitrogen, potassium, etc., and the further assimilation by the host of food suitable for the fungus would cease. Even the fact that the rust thrives for a time upon a leaf so nourished does not preclude the possibility of a partial protein-related diet. In an ordinary detached leaf there undoubtedly exists sufficient amounts of nitrogen, potassium, etc., within the cells to insure the normal development of the rust for a considerable period. The experiments of Ward (49) and Mains (21) showed that even when the host plant was starved with respect to certain mineral elements, infection could still take place.

To induce teliospore formation upon the leaves of *Taraxacum*, as is shown in the results of the experiment, it was necessary to make several changes in the medium upon which the leaves were floated. They were first floated upon distilled water, then immediately after inoculation changed to sugar solution for 16 days. At the end of this time they were transferred to distilled water for five days, after which they were returned to the sugar solution.

At the time of the transfer from sugar to distilled water the rust was producing an abundance of uredinia. This would mean that a great amount of food was being taken up from the sugar solution by the leaf and appropriated by the fungus. The sudden change to distilled water cut off this continuous food supply and stimulated the fungus to initiate the produc-

tion of the telial generation. However, due to the insufficient supply of stored food both in the leaf cells and in the mycelium, the fungus could not carry through to completion the formation of the telial stage. This was shown when the leaves were allowed to remain on distilled water at this stage of the experiment. If, after a period of five days the leaves were again transferred to the sugar solution, adjustments already initiated due to the stimulus were carried over and the fungus completed the formation of the telial stage. Soon after, the effects of the addition of the nutrient to the leaf cells again caused the fungus to resume uredinial production.

The Light Relations

With regard to the influence of light upon the growth and development of the rust mycelium, little need be said, for Fromme (12), Mains (21), and the writer (50) have shown that, in the organisms employed by them, the main influence is exerted indirectly through the host. We can consider that any results obtained from the experiments reported in this paper represent the effects of light acting not especially upon the fungus, but mainly and, perhaps only, through the host.

In five of the organisms studied, namely, *Uromyces appendiculatus*, *Puccinia suaveolens*, *U. trifolii*, *U. polygoni*, and *P. sorghi*, it was possible to inhibit the uredinial stage and hasten the time of telial formation by placing the infected plant in the dark for varying periods of time. The time of appearance of teliospores varied up to a certain limit directly with the length of time the host was left in darkness. Since the temperature was practically the same as for checks in the light, the effects produced could only have been due to the decrease in photosynthetic activity of the host, with the resulting partial starvation of the fungus.

It must not be assumed, however, that mere starvation and injury to the host in all cases will guarantee the appearance of the telial generation. It is undoubtedly true that the diminution of food will initiate the formation of such a spore stage, but in all cases the fungus must have access to enough nutrient to carry through to completion the reaction to such a stimulus. This was shown in the case of corn rust. When the host was subjected to total darkness for either three or four days, both host and parasite died before the telial generation was formed. Two days, however, under the same conditions produced the necessary "starvation" stimulus and, when the host was again removed into the light to the greenhouse, it was capable of synthesizing just enough food to enable the fungus to complete the formation of the telial stage. If the metabolism of the host and fungus are not too greatly disturbed, it should be possible, in some cases, to induce uredinial formation again following the telial stage. This is what actually occurred in the case of *Uromyces polygoni*. When plants

P1-5 and P5-10 were removed from the cold, dark room (7° C.), and the dark room (19° C.), respectively, after having been there for four days, the telial stage was first produced; then, under the influence of the favorable conditions of the greenhouse, the uredinial generation was again produced. When, for the second time, the plants were subjected to the same conditions of light and temperature as before, the fungus proceeded to produce the telial stage again. This time, however, plants P1-5 were not able to respond to the resumed favorable conditions and the fungus continued to produce the telial stage until the death of the infected leaves. On P5-10, which had not been so severely affected, uredinial production was resumed for a short time before finally the telial stage appeared.

Several of the other rusts reacted in a similar fashion, and the results appear so positive that discussion is not deemed necessary to point out further the direct connection between the presence or absence of food and the type of spore produced.

Moisture Relations

The experiments conducted relative to moisture control seem to show that, in certain rusts at least, the amount of moisture available for the host and parasite, be it soil or atmospheric moisture, exerts an influence on the type of spore produced by the rust parasite.

The conclusions drawn by the writer from the results of experiments conducted with asparagus rust are perfectly in accord with those of Smith (40), as far as the assumption goes that teliospores are produced when moisture conditions, be it soil or atmospheric, are unfavorable for the further vegetative development of the fungus. The lack of agreement comes in the interpretation of the manner in which the moisture affects the fungus.

Smith assumes that an abundance of soil moisture favors the host by giving it increased vitality and resistance with the result that the fungus is retarded and forced to form the telial stage. This is clearly at variance with the findings of such workers as Arthur (3), Stakman (41), Ward (48), Stakman and Levine (43), *et al*, mentioned at the beginning of the discussion. However, as Smith's conclusions are based on field observations, it appears to the writer that there are too many unknown factors which may have entered in to ensure the absolute accuracy of his interpretations.

Sheldon (37, 38), was forced to disagree with Smith's interpretations, for he concluded that, as a rule, conditions favorable for the development of the host were also favorable for the development of the fungus. The same conclusions were reached by the writer, with an added feeling of assurance that an abundance of soil moisture not only increases the vitality of the host but also lengthens the period of uredinial production by the rust.

When plants A5-6 were placed in the greenhouse and kept well watered, they continued to produce the uredinal stage for a week longer than did plants A1-5, which were deprived of their soil moisture.

If the conclusions of Smith be correct, then the presence of abundant soil moisture should have rendered the host an unfavorable medium for the fungus and the telial stage should have appeared sooner than on plants A1-5. This, however, was not the case. On the contrary, the results seemed to show that the abundance of soil moisture exerted a favorable effect on the host and also on the fungus, due to the increased ability of the former to produce an abundance of food.

With respect to the action of atmospheric moisture, Smith (40) asserts that "atmospheric dryness checks aecidial development and uredo-development, and changes to a production of teleutospores in the sori already formed, without regard to season or conditions of the host plant. With moisture, uredospore formation begins again at once."

It is difficult to understand how such a statement can be positively made, for such an assertion disclaims any influence that the living host might exert at the same time. Unless Smith can show that the asparagus rust parasite fails to maintain that close association with the host which is common to so many of the rusts, he must admit that the condition of the host should play an exceedingly important rôle. Can he maintain that the host, under the influence of high humidity, is, physiologically speaking, the same host upon which the telial stage was being produced? Is it not true that the increase in humidity would tend to lessen the transpiration and evaporation from the infected stalks with the result that the metabolic rates of both the fungus and the host would remain lower and the cells of the host remain alive for a longer period of time? Would not this in itself affect the food relations of the fungus?

Resistance of the Host

Fromme and Wingard (13) found a relationship between the resistant varieties of bean and teliospore formation. When certain resistant varieties of bean were inoculated, teliospores, or mixed sori, made their appearance immediately.

In working with *Puccinia coronata* Corda, on *Avena sativa* L., Parker (31) observed that frequently the telial stage appeared on seedling leaves of resistant varieties, while on the susceptible varieties it did not occur.

In table 3 it is seen that on the Scarlet Wax variety of bean, which is resistant to rust, teliospores were formed 15 days after inoculation, or only three days after the first appearance of the uredinal stage. In some cases the teliospores appeared without being preceded by this stage.

It is impossible to analyze all the possible factors in resistant and susceptible plants, as no one has yet satisfactorily explained what the phenomenon of resistance actually means. The cytology of infection, both in resistant and susceptible plants, has been investigated by several, among whom are Ward (47, 48), Evans (11), Stakman (41, 42), and Allen (2). In all cases they have shown that, when a rust infects a host, vigorous development takes place without any immediate serious injury to the host. In fact, the host cells seem to be stimulated to greater activity for a while. In resistant hosts, however, the host cells in the immediate vicinity of the haustoria are killed and the fungus makes little headway. The extent to which this killing takes place seems to depend upon the degree of resistance of the host. Whether, as Marrayat (24) has declared in the case of *Puccinia glumarum*, the fungus starves as a result of the death of the host cells, it is difficult to say; but, at any rate, from all the cytological evidence presented, it is certain that the fungus in a resistant host is not able to establish a satisfactory food relationship with the host. Such being the case, it is entirely possible that this condition of partial starvation would immediately stimulate the production of teliospores as was seen in the bean.

Since, as has been pointed out in all the above experiments, the appearance of the telial stage is an index of some metabolic disturbance within the host, it is entirely within reason to suppose that the early appearance of the telial stage on a resistant plant is due to the inability of the fungus to establish a satisfactory food relationship with the host.

SUMMARY

1. From the results of the experiments herein described, it appears that all the rusts studied are directly dependent upon the photosynthetic activity of the host. Any single factor or set of factors, such as light, temperature, and moisture, or, as in the case of climate, a complex of these factors, may so influence and do influence the metabolism of the host, that the fungus reacts by changing from the uredinial to the telial generation, or, under proper manipulation, in the reverse direction.

Results obtained in the greenhouse

2. Nine species of rusts were cultivated in the greenhouse and experiments conducted to show that teliospore production can be controlled.

3. With the exception of *Puccinia triticina* on *Triticum vulgare*, teliospores were formed in all the rusts when the host plants were placed under various environmental conditions unfavorable for their metabolism.

4. In seven of the rusts, teliospores were produced when the infected hosts were placed in darkness at 7° C., or in darkness at 19° C.; in the

former environment teliospores appeared several days sooner than in the latter.

5. The conditions of light and temperature necessary for production of the telial stage vary with different hosts. Such conditions appear to be governed largely by the nature and habits of growth of the host.

6. In three of the rusts, teliospores were produced when the infected hosts were slowly deprived of water, the amount and general procedure varying with the nature and habit of the host.

7. Resistance of the host plays a part in the production of teliospores. In the case of bean rust (when the host was partially resistant) the telial stage was formed several days earlier than when it was susceptible. In some cases the uredinial generation was completely inhibited and teliospores were the first structures to appear following infection.

8. By removing the growing point of the bean plants immediately following inoculation and thus preventing the withdrawal of food from the primordial leaves, the time of teliospore formation was postponed several days.

Results obtained in petri dishes

9. A method was devised by which detached leaves or portions of leaves of the host plant could be infected and floated on water or nutrient solutions in petri dishes. By this method infections were obtained regularly and in some cases they exceeded in amount those obtained on plants in the greenhouse.

10. Ten rusts, including the nine used on potted plants, have been cultured by the above method. The additional rust was *Puccinia orbicula* Pk. and Clint. on *Prenanthes alba* L.

11. Urediniospores and teliospores were produced in eight of the rusts which were cultured in dishes, the type of spore formed depending on the treatment to which the leaves of each host were subjected.

12. The rusts in which the telial generation was produced most easily in the greenhouse were also the ones which responded most readily to experimental methods in dishes.

13. When infected bean leaves were transferred at the time of uredinial formation from a 7 per cent cane-sugar solution to distilled water, if the infection were not too heavy, teliospores were produced much sooner than on leaves which were allowed to remain on the sugar solution continuously.

14. On infected bean leaves, grown on distilled water, teliospores appeared greatly in advance of those on leaves which were better nourished.

15. The heaviest infection on bean leaves was obtained by transferring the leaves from distilled water to sugar solution six days after inoculation.

16. Starch tests made on bean leaves at the time of uredinial and telial formation showed an abundance of starch at the time of uredinial formation and a paucity of starch during the production of telia.

17. In the case of most of the rusts cultured in petri-dishes, teliospore formation was stimulated by any one of the three following methods: (a) starvation of the host with the later addition of a rich food supply, (b) sudden transfer of the host leaves from a well-fed condition to a state of starvation, (c) continuous supply of food with the gradual dying of the host cells.

18. In two of the rusts the telial generation was induced only by transferring the leaves from the sucrose solution to distilled water and back again.

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A MOSAIC DISEASE OF GLADIOLUS¹

LOUISE DOSDALL

While examining Gladiolus corms for various diseases in 1925 several Primulinus hybrid corms were found with a peculiar deformed appearance suggesting cucumber nubbin. The normal corm in this variety is plump with a smooth, clear, yellow epidermis, whereas the abnormal corms were distinctly warty and the color was mottled. On the elevated areas the epidermis was yellowish, while in the depressed areas it was paler and sometimes greenish.

Several of these corms were planted in pots in the greenhouse, together with several normal corms. Certain symptoms quite characteristic of mosaic diseases developed in the plants from the peculiar corms. The leaves pushed out to about one-half their normal length, then ceased to develop further. They seemed somewhat thicker and stiffer than normal leaves and were mottled much like sugar cane leaves affected with mosaic. The plants from the normal corms were taller, the leaves were dark green, and there was no mottling. An attempt was made to produce the disease in healthy plants by extracting the juice from mottled leaves and rubbing it on the surface of uninjured young growing leaves and on young leaves which had been pricked, and by injecting the juice with a needle. Only negative results were obtained.

In the summer of 1925 the grower from whom the corms were obtained had reported that, while his plants seemed to be free from disease, there was a very decided difference in the appearance of the plants from his old corms and those from new corms which he had obtained from the east. With the same variety there was a sharp line marking where the one set of plants stopped in the row and the others began. The new plants were much darker green and seemed more vigorous than the old ones.

In the spring of 1926, while going over a lot of miscellaneous corms, many of the peculiarly warted ones were noted. Fifty of these warted corms were selected. As a check, fifty smooth corms as nearly similar in size as it was possible to select them were taken. Both lots were treated with formaldehyde and planted in parallel rows. Ninety-four per cent of the smooth corms and only seventy per cent of the checks produced plants. The plants from the warty corms were decidedly less vigorous than the others, and nearly all of the plants developed the same type of mottling

¹ Published with the approval of the Director as Paper No. 732 of the Journal series of the Minnesota Agricultural Experiment Station.

in the leaves as did the plants which had grown in the greenhouse. Some of the plants in the check row also developed mottled leaves. This mottling of the leaves was found to be extremely common in all gladiola plantings visited during the summer.

The plants from the warty corms blossomed considerably earlier than the others. The first blossom opened on July 12 in the one case and on July 26 in the other. Of the plants from warty corms, 48 per cent produced blossoms; of the checks, 95 per cent. Most of the plants in this experiment belonged to the variety Gretchen Zang. In many cases the mottling was very prominent on the peduncle and bracts of the inflorescence and extended also into the petals (Pl. II, A). The normal pink color of the petals was broken with greenish white.

There was a decided difference in the yield of corms from the two lots. Very few cormels were produced by the diseased plants and the corms were only one half the size of those from the check row. Both lots of corms were again planted in parallel rows in 1927. Fourteen per cent of the progeny of the original selection of warty corms produced plants. Fifty-six per cent of the progeny of the original check produced plants. Practically all of the plants in the diseased row were mottled. Most of the plants in the other row also showed signs of the disease to a greater or less extent. The second year (1927) only one plant in the diseased row blossomed. This plant proved to belong to the variety Willie Wigman. The leaves were dark green and there was no evidence of mottling.

The same type of disease has been observed in various varieties. In the 1926 planting of Pride of Goshen, many of the plants were badly stunted, the leaves were mottled, and the malformations of the inflorescence were pronounced. The peduncle was very much shortened, the individual flowers were closer together and smaller than the normal, and the petals were almost greenish. Twenty-six corms from the planting were replanted in 1927. Of these, only seven produced plants. Only one of the seven plants produced a normal blossom. A planting of the variety Alice Tiplady next the Pride of Goshen in 1926 was particularly vigorous and no signs of the mosaic were noted. When the corms were cleaned during the winter, however, the buds under the sheaths were found to be covered with the cotton aphid, *Aphis gossypii*. In 1927 many of the plants from these corms were mottled. The symptoms on the leaves were particularly striking (Pl. II, B). This is a *Primulinus* Hybrid type with flowers far apart on the central axis. On plants in which the infection extended into the blossom the shortening of the peduncle and bunching of the flowers in the inflorescence was very marked. The breaking of the color in the blossom was very pronounced also. Aphids were abundant on the plants until the

first leaves were about half grown. Later in the season none could be found. Plants of the variety Schwaben have also been badly infected. The malformation of the corm is very marked (Pl. II, C), as is also the stunting of the plants and the mottling of the leaves and flowers (Pl. III, A).

The peculiar wartiness of the corms is very marked in the variety Gretchen Zang (Pl. II, D). On cutting sections through such warty corms it was found that the abnormality in structure extended through the entire corm. From freehand sections it was found that this structure was due to the fact that there were areas of large cells filled with starch grains and areas of small cells with no starch grains quite similar to the green and chlorotic areas in mosaic infected leaves. In the normal corm the starch is distributed evenly throughout the corm. This difference is shown very beautifully by staining slices of corms with iodine (Pl. II, E). Similar results were obtained with Schwaben and Primulinus Hybrid corms.

These observations indicate quite definitely that the abnormal condition is a degeneration disease of Gladiolus. The symptoms are very marked at all stages in the life of the plant; on the corm, the leaves, and the inflorescence; and are characteristic of the symptoms of mosaic diseases in other plants. The disease is transmitted through the corm from one generation to the next. Eventually the plant is killed. Probably the disease is transferred from one plant to another by sucking insects although this has not yet been demonstrated to be true.

UNIVERSITY FARM,

SAINT PAUL, MINNESOTA.

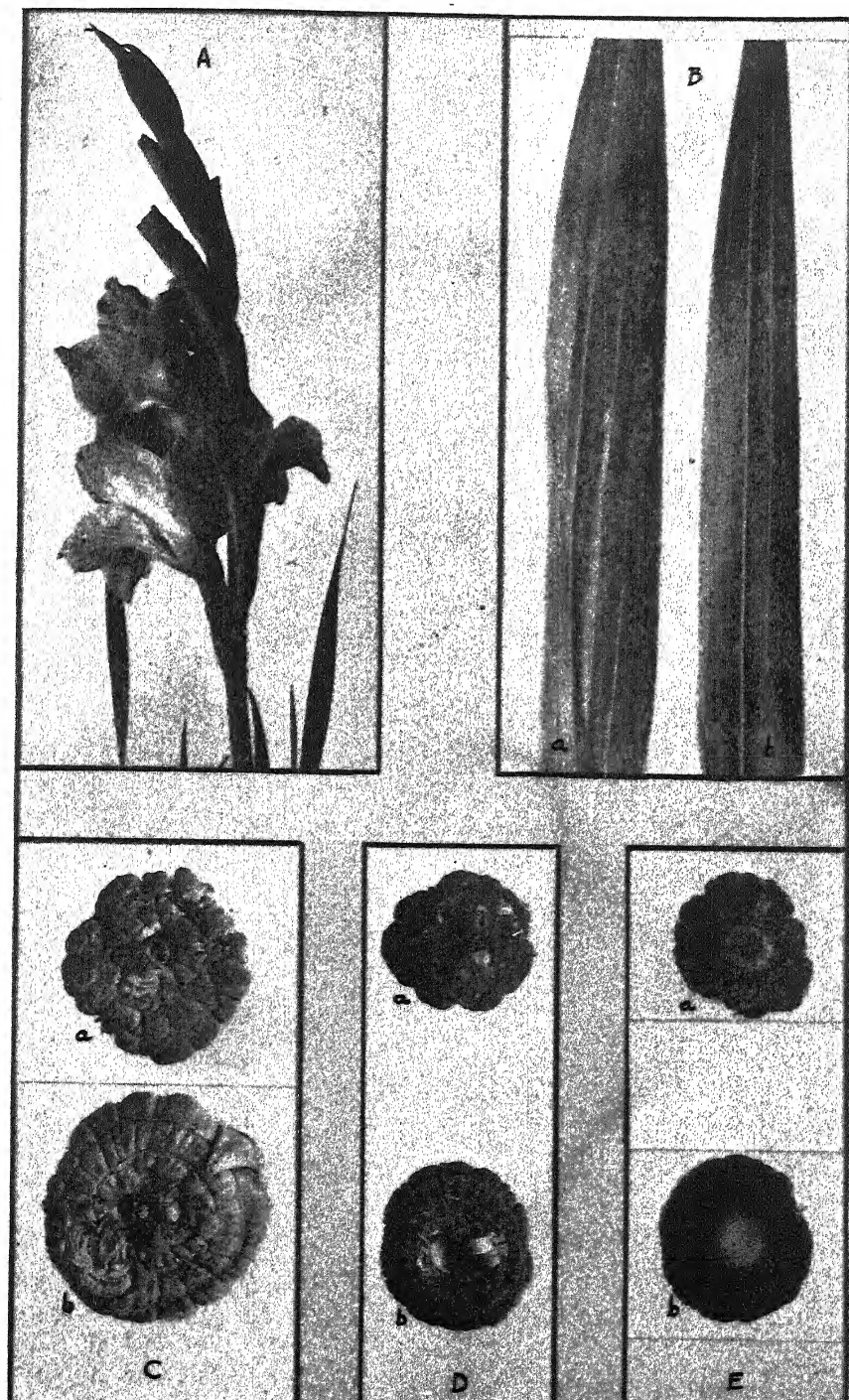
EXPLANATION OF PLATES

PLATE II

- A. Inflorescence of the variety Gretchen Zang showing the breaking of the color in the blossom.
- B, a. A diseased leaf of the variety Alice Tiplady showing the characteristic mottling.
b. A normal leaf of the variety Alice Tiplady.
- C, a. A diseased corm of the variety Schwaben.
b. A normal corm of the variety Schwaben.
- D, a. A diseased corm of the variety Gretchen Zang.
b. A normal corm of the variety Gretchen Zang.
- E, a. A section of a diseased corm of the variety Gretchen Zang, stained with iodine.
b. A section of a normal corm of the variety Gretchen Zang, stained with iodine.

PLATE III

- A. Inflorescence of the variety Schwaben showing the characteristic mottling on the peduncle and bracts and to some extent the crowding of the flowers.
- B. A normal inflorescence of the variety Schwaben.





WASHINGTONIA PALM LEAF SPOT DUE TO CYLINDROCLADIUM MACROSPORUM N. SP.

C. D. SHEERAKOFF

INTRODUCTION

In September, 1916, the writer found in a glass-covered bed, in central Florida, seedling leaves of *Washingtonia robusta* Parish, severely affected with numerous, small, dark leaf spots with translucent borders. On the spots was found a Hyphomycete which was readily recognized as belonging to Morgan's¹ genus *Cylindrocladium* and very similar, except for a marked difference in the size of the conidia, to his *C. scoparium*, the fungus found on a dead pod of *Gleditschia triacanthus*. The species was the only one known at that time.

At the end of 1916 the writer found that Dr. L. M. Massey² was working with a *Cylindrocladium* isolated from crown canker of roses and exchanged cultures with him. The conclusions of the writer's examination (Fig. 1, D) of Massey's rose fungus agree with his, that it should be considered the same as Morgan's *C. scoparium*. Dr. Massey also found that Ellis and Everhart's³ fungus, found on dead leaves of the papaw tree, and described under the name of *Diplocladium cylindrosporium* E. and E., is also *C. scoparium*. In 1918 Dr. P. J. Anderson⁴ published his work on rose canker, agreeing with Massey's conclusions regarding the identity of the fungus. However, in association with *C. scoparium* he found also another fungus of the genus and, because of its much smaller conidia and of some other differences, described it as a new species, *C. parvum*.

In 1919 Professor H. E. Stevens, then plant pathologist of the Florida Agricultural Experiment Station, collected some specimens of leaf spot on mature *Washingtonia* palm growing on the Station grounds and, because he thought that the spots might be similar to those previously found, gave them to the writer. Examination showed that the fungus was in all main characters, except for the size of conidia, exactly like the fungus found on the seedling leaves in 1916.

¹ Morgan, A. P. Two new genera of Hyphomycetes. Bot. Gaz. 17: 190-192. 1892.

² Massey, L. M. The crown canker of the rose. Phytopath. 7: 408-417. 1917.

³ Ellis, J. and M. B. Everhart. New species of fungi from various localities, with notes on some published species. Bul. Tor. Bot. Club 27: 58. 1900.

⁴ Anderson, P. J. Rose canker and its control. Mass. Agr. Exp. Sta. Bul. 183: 9-46. 1918.

In 1920 the writer left Florida and thus had no opportunity to complete the work with the fungi. However, in Tennessee, in 1922, he isolated a *Cylindrocladium* from the crown of a yellowish plant of red clover brought in by Earl Felix, a student. The fungus was found to be the same as *C. scoparium* in general cultural and morphologic characters and in size of the conidia. Finally, in 1924 and 1925 the writer isolated a *Cylindrocladium* from dark brown spots on specimens of roots of apple seedlings received from a western state. The apple-root fungus also appears to be *C. scoparium* (Fig. 1, E).

Representative members of the genus *Cylindrocladium* were thus found on a wide range of hosts covering a large territory, so that the fungi can hardly be considered as rare. But evidently they are usually overlooked by mycologists and plant pathologists. To call their attention to the subject is the purpose of this brief paper, which, in its present state, is merely a series of notes. In spite of the writer's wish to round up the subject and present it in a more finished form, it became apparent that the paper should be published without further delay, because the writer finds that he will not have sufficient time in the near future to give to the subject.

DESCRIPTION AND ECONOMIC IMPORTANCE OF THE WASHINGTONIA PALM LEAF SPOT

The spot is from less than $1\frac{1}{2}$ mm. to over 2 mm. in diameter, round to oblong, mostly with smooth though sometimes angular outlines, dark greenish-brown, with a narrow translucent border. The surface is smooth or, in damp air becomes covered with a thin, whitish, somewhat coarsely powdered growth of the fungus. The spots are about the same in appearance (Fig. 1) whether they occur on the simple leaves of the palm seedling or on the fan-shaped leaves of a mature palm, although in the particular specimens of the fungus collected from seedlings in 1916 and from the mature palm leaves in 1919 there were noticeable differences in size of the conidia.

The writer's observations indicate that the disease spreads rapidly and causes noticeable damage to the leaves only when there is abundant moisture in the air; otherwise the disease remains almost inactive for quite a long time and thus seems to be of no economic importance even in Florida's rainy season in the summer. The only time the disease was observed to do severe damage was in a glass-covered bed filled with palm seedlings where the ventilation was very poor.

The Fungus

A. P. Morgan's descriptions of the new genus *Cylindrocladium* and of the new species *C. scoparium* are as follows:

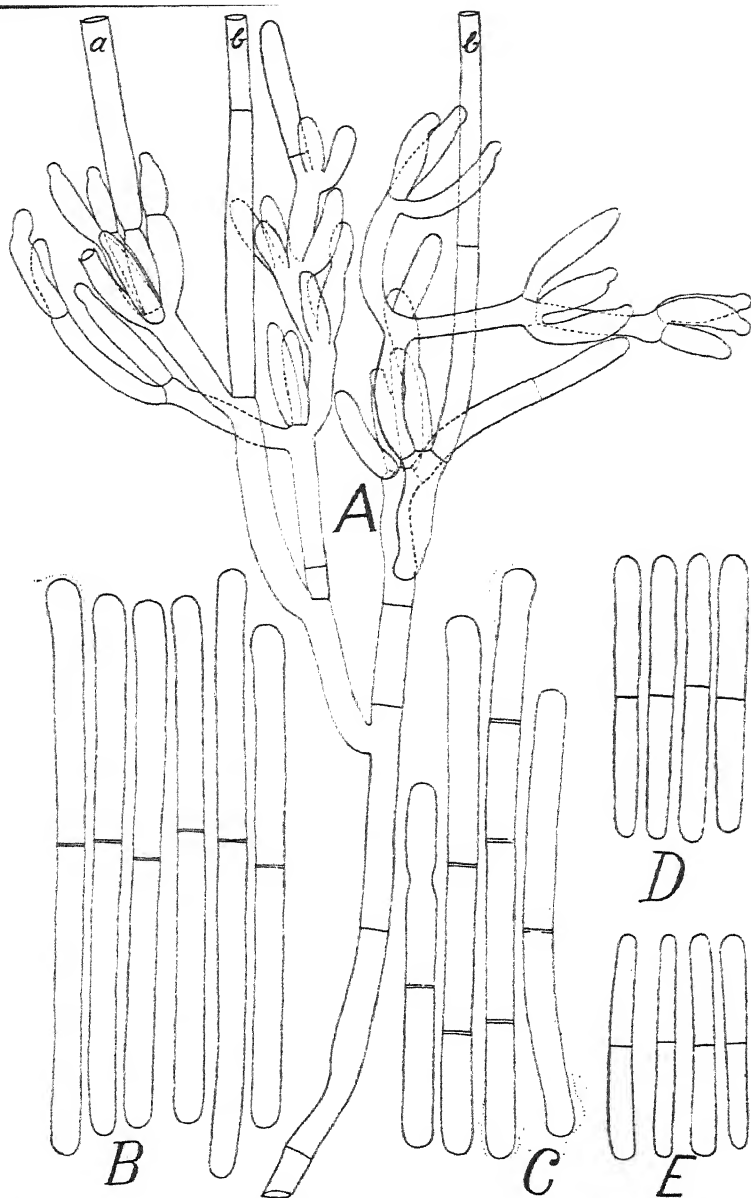


FIG. 1. A-C. *Cylindrocladium macrosporum* n. sp.: A. A conidiophore; a. Lower part of the conidium still attached to the sterigma; b. Lower part of long terminal hyphae which extend farther up from the point at the top of the drawing to from 100 to 200 μ and are slightly swollen at the upper end; B. Typical conidia; C. Conidia showing deviations from the type either in the shape or in the septation; the dotted lines about some of the conidia in B and C show the visible lines of a water soluble substance with which the conidia are covered. D. Conidia of *C. scoparium* Morgan, from Massey's culture of the rose canker fungus, on cornmeal agar 14-day old plate. E. Conidia of *C. scoparium* Morgan, from the author's isolation from root spot of apple seedling, a young tube culture on potato agar. A-D are from camera lucida drawings; E is a reconstruction from a photomicrograph. Magnification 750 times.

“*Cylindrocladium* gen. nov.—Sterile hyphae creeping, branched; fertile hyphae erect, forked or trichotomously branched, the sporophores in pairs or threes at the extremities of the branchlets and cymosely arranged; spores solitary, cylindric, 1-septate, hyaline.

“*C. scoparium* n. sp.—Effused, thin, flocculose, white; sterile hyphae creeping, slender, indistinct; fertile hyphae thick, erect, hyaline, septate, cymosely branched above, the sporophores short, disposed in pairs or threes at the extremities of the branchlets, each producing a single spore at the apex; spores cylindric, tapering slightly downward, 1-septate, hyaline, obtuse at each end, 40–50 μ in length, 4 μ at the apex and 3 μ at the base.

“Growing on an old pod of *Gleditschia triacanthus*. The sterile hyphae abundant enough, but they are fine, slender threads creeping close to or beneath the surface; the fertile hyphae have a simple septate stem 5–7 μ in thickness and are dissolved above into a level-topped cyme of branches; their height, exclusive of the spores which easily fall off, is 125–150 μ .”

The description and the figure of the fungus, when compared with the fungus found by the writer on the Washingtonia palm leaf spots, indicate that the two are very much alike in all main morphologic characters, except for the size of the conidia, which are much longer and somewhat wider in the latter fungus. The fungus from the Washingtonia palm was readily isolated in pure cultures, by a dilution of the conidia found on the spots and by planting in cornmeal agar plates small bits of the leaf tissues taken from the spots, which were washed for 10 to 12 seconds in 1:1000 mercuric chloride solution and rinsed afterward in sterile water. It may be of some interest to note that the fungus for a few months after its isolation produced an abundance of conidia on cornmeal and potato agars and on steam-sterilized bean pods. Later, however, the fungus lost the ability to produce spores, and it became necessary to renew the vigor of a culture by host inoculation. After reisolation, the fungus sporulated again for several months.

In addition to the conspicuously larger size of conidia, there are also some other morphological and physiological characters, as well as in relationship to the host, in which the fungus differs from *C. scoparium*, sufficiently, the author believes, to be considered as a separate, new species which is described here as follows:

Cylindrocladium macrosporum n. sp.—Fig. 1, A–C. Mycelium, conidiophores, and conidia hyaline; conidiophores erect, usually with one, two or more side branches each ending with two, three or more slightly curved, cyme-forming sterigmata; some of the branches, usually terminal, instead of producing sterigmata give rise to very long hyphae slightly swollen at the tip; on the sterigmata are produced singly long, cylindric, straight, obtuse, often slightly swollen at the ends, somewhat narrower at the base, 1-septate conidia which are apparently glued together with a water soluble, colorless substance so that when placed in water they easily separate. The

conidia in strain 1, from seedling palm leaves, measure $103.80 \times 5.35 \mu$ ($71.00-131.00 \times 5.15-5.94 \mu$), and those in strain 2, from leaf spot of mature palm, measure $82.00 \times 5.25 \mu$ ($60.00-105.00 \times 4.50-6.20 \mu$). The fungus in culture, especially in old cultures, usually produces chlamydospores in clusters and chains and some minute, submerged, brown sclerotia, though the latter were never observed to be so numerous and dark as in *C. scoparium*. The color of the species, on the same media, is noticeably lighter than that of *C. scoparium* and is free from the red color of the latter species observed in some cultures.

Habitat.—Found in Florida on leaves of *Washingtonia robusta*, causing leaf spots.

The size of the conidia of the different isolations of *C. scoparium* and of the species of the *Cylindrocladium* from the *Washingtonia* palm is as follows:

C. scoparium from *Gleditschia triacanthus*, $40-50 \times 3-4 \mu$, (Morgan).

from *Asinina triloba*, $40-50 \times 4-5 \mu$, (Ellis and Everhart).

from *Rosa* sp., $48.30 \times 4.13 \mu$ ($36-55 \times 3.3-4.51 \mu$), (Massey).

from *Rosa* sp., $39.20-48.80 \times 4.03-5.10 \mu$, (Anderson).

from *Malus* sp., $42.5 \times 4.2 \mu$, (Sherbakoff).

Average size of conidia of *C. scoparium*..... $45.0 \times 4.2 \mu$.

C. macrosporum from *Washingtonia robusta*, strain 1 from seedlings:

40 conidia from 11-day culture on cornmeal agar plate— $102.00 \times 5.20 \mu$ ($75.0-125.0 \times 4.9-6.0 \mu$). Measured in 1916.

20 conidia from old bean-pod culture— $102.40 \times 4.90 \mu$ ($92.0-118.0 \times 4.5-5.2 \mu$). Measured in 1916.

100 conidia from 12-day culture on cornmeal agar plate— $102.00 \times 5.20 \mu$ ($74.0-123.0 \times 4.9-5.5 \mu$). Measured in March, 1917.

40 conidia from 11-day culture on cornmeal agar tube— $103.80 \times 5.35 \mu$ ($71.00-131.00 \times 5.15-5.94 \mu$). Conidia about 5.25μ wide at narrow end and 5.47μ at broad end. Measured in October, 1917.

Average size of the conidia of strain 1— $102.50 \times 5.20 \mu$.

C. macrosporum from *Washingtonia robusta*, strain 2 from mature leaves:

50 conidia from leaves— $84.00 \times 5.25 \mu$ ($60.00-105.0 \times 4.5-6.2 \mu$). Measured October, 1919.

10 conidia from 5-day culture on cornmeal agar plate—
78 μ (75–80 μ) in length. Measured in November,
1919.

Average size of the conidia of strain 2—83.00 \times 5.25 μ .

Briefly the average size of the conidia of different *Cylindrocladia* known at present are: *C. parvum* about 17.00 \times 2.50 μ ; *C. scoparium* about 45.00 \times 4.20 μ ; *C. macrosporum*, strain 1—102.50 \times 5.20 μ , strain 2—83.00 \times 5.25 μ .

PATHOGENICITY

Massey and also Anderson definitely proved the ability of *C. scoparium* to produce the cankers on the greenhouse roses and showed the economic importance of the disease and devised certain methods of its control.

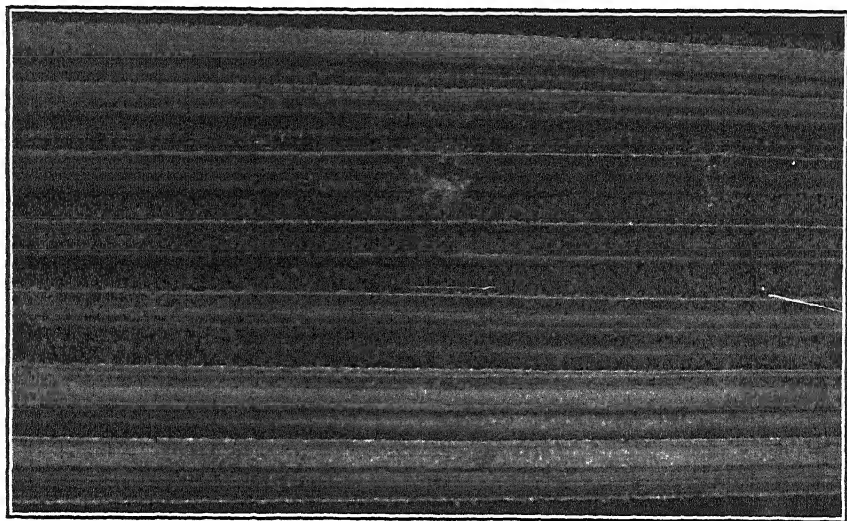


FIG. 2. A photograph of a part of the Washingtonia palm seedling leaf, showing the spots produced by an inoculation with a pure culture of *Cylindrocladium macrosporum*. The large spot—toward reader's left and slightly upper side of the photo—is where a bit of the inoculum was placed; the other spots arose evidently from the infections by the conidia scattered from the culture. About 1½ times natural size.

The writer, in March, 1917, made a few inoculations of the Washingtonia palm potted seedlings. The seedling leaves were sprayed with sterile water and inoculated with small bits of pure fungous cultures with many conidia from cornmeal agar. They were then covered with bell jars. For checks, several other palm plants were watered and likewise covered but not inoculated. Four days later, on the leaves of about half of the plants,

there appeared spots similar to those originally found (Fig. 2) and from which the fungus was isolated. Small bits of the tissues from the spots were then cut out, disinfected 10-12 seconds in 1:1000 solution of corrosive sublimate, rinsed in sterile water, and plated on cornmeal agar. On the plates pure growths of the *Cylindrocladium* developed, which in all characters, size included, were identical with the originally isolated fungus.

Acknowledgment. The work during 1916-1920 was done at the Florida Agricultural Experiment Station, Gainesville, Florida, where the illustrations, except part E. fig. 1, also were made. The paper is published with the permission of the Director, Wilmon Newell.

TENNESSEE AGRICULTURAL EXPERIMENT STATION,
KNOXVILLE, TENNESSEE.

CONSTANT TEMPERATURE AND HUMIDITY CHAMBERS

JAMES JOHNSON¹

Six years ago the writer described a set of air-control chambers suitable for conducting experiments on the relation of environment to plant disease. Shortly afterward the chambers were moved to another greenhouse to secure refrigeration and at the same time several modifications were made which proved to be decided improvements on the earlier apparatus. These chambers have now been in almost continuous operation for five years with no modification and few repairs and are consequently deemed worthy of description for the benefit of those who contemplate the construction of similar apparatus.

The importance of studies on the relation of temperature and humidity to plant disease is fully recognized. The initiation of such investigations is largely limited by the difficulty of constructing equipment giving satisfactory control and by the cost of such equipment. It seems quite apparent, however, judging by the number of pathologists and physiologists expressing interest in air-control chambers, that the problem of controlled environment in relation to plants will receive increasing attention in the future. Since the construction of the chambers in question, two other units have been set up in the Wisconsin laboratories (2) (10), which are, however, more or less different from the type herein described. Air-control units for the growing of plants are also being used in several other American institutions, notably the Illinois Agricultural Experiment Station (3) (4), the Boyce Thompson Institute for Plant Research (1), and the Nebraska Agricultural Experiment Station (8).

GENERAL PLAN OF EQUIPMENT

The three chambers of the air-control series to be described are each located in separate but contiguous greenhouse units in which the temperature is thermostatically regulated to correspond roughly to the temperatures of the respective chambers which they contain. The purpose of this arrangement is to eliminate condensation of moisture on the glass walls of the chambers, which may occur when large differences in temperature exist between the surfaces exposed in very humid air. This arrangement also aids in reducing the amount of necessary refrigeration or heating, as the case may be, inside the chambers.

¹ Wisconsin Agricultural Experiment Station in cooperation with the Office of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

The three chambers are identical in construction, except for the amount of refrigeration area contained in each. The chamber usually used for the medium degrees of temperature contains approximately 24 linear feet of one and one-half inch galvanized iron pipe. The chamber usually used for the lower temperatures contains 48 feet of pipe, which is in two units however, so that only 24 feet need be used if desired. The high temperature chamber can be operated with 27 feet, 24 feet or 3 feet of refrigeration pipe as desired (Fig. 1). The refrigeration can, of course, be cut off completely from any one or all of the chambers. The variation in length of refrigeration pipe is not only useful in permitting the use of wide ranges of temperature, but is important also in meeting the influence of seasonal conditions on the temperature inside the greenhouses. It is practically impossible even with a large refrigeration surface to combat the highest temperatures of summer. The chambers are therefore not ordinarily operated during the summer months for plant cultures, but with the sunlight shut out we have operated them for other purposes, such as the study of environment on the curing of tobacco. With the present system of refrigeration, constant temperatures as low as 10° C. can usually be secured during eight months of the year in a chamber containing 48 feet of refrigeration pipe.

The refrigeration in the chambers is furnished in the present instance by a 10-ton Audiffren machine (General Electric Company). This machine furnishes refrigeration for several other purposes, and a considerably smaller machine would, of course, suffice for the operation of the amount of refrigeration surface described. Refrigeration is such an important item of cost in the construction of chambers that it should be stated that the amount required is dependent upon a number of factors. Much can be accomplished in the way of constant temperatures during the winter months in the northern climates without any refrigeration. Where temperatures below about 18° C. are required, however, over a considerable period of time, artificial refrigeration is necessary. At mid-day, with strong sunlight, shading the chambers is often necessary, even with refrigeration, to hold the temperature down to normal, and this practice needs to be considered particularly in chambers lacking required refrigeration capacity. As it is ordinarily true that the critical temperature for plant diseases lies above 20° C., it may suffice in many cases to have refrigeration in only one chamber. For this purpose, a small machine of the type used for household refrigerators may sometimes prove sufficient. In general, the refrigeration system necessary will depend upon the prevailing temperatures at the season of the year at which it is desired to operate the chambers, and upon the host or the disease which is to be studied.

The supply of humid air for the chambers is furnished by a spray of water heated to the desired temperature. Since a spray of water operates continually for each chamber, it is important to have an adequate and continuous supply of warm water. In our units, this is furnished by a 60-gallon tank, in the water-pressure circuit, heated with high-pressure steam and regulated by a thermostat. The Instanto Steam Water Heater (North Manchester, Ind.) has proved very satisfactory for our purposes. This system also furnishes water for other air-control units, and a tank of half this capacity would be sufficient for the operation of the chambers described in this paper. Before high-pressure steam was available for this purpose, a 40-gallon, gas-heated water tank furnished the hot-water supply in a satisfactory but less reliable manner.

The refrigeration system and hot-water tank are located in a building adjoining the greenhouses through which the brine and hot water are piped to the chambers.

It is also important to install a separate line of electric current of sufficient capacity from the fuse box to the chambers, upon which no additional electric load can be added. The overloading of the electric line is likely to cause interruptions in the best operation of the control units. The heaters and motors of the chambers to be described require a line carrying approximately 60 amperes of electricity. All the apparatus in our chambers is operated with 110 volt, single phase, 60 cycle alternating current.

CONSTRUCTION OF THE CHAMBERS

The chambers are four-foot cubes with three sides and bottom of wood and the top and one side of glass (Fig. 1). This arrangement naturally sacrifices a considerable amount of light which might otherwise be available if the top and all sides were of glass construction. In actual practice, however, there does not seem to be any decided advantage in an all glass chamber. The normal illumination is greatly reduced in either case, and during periods of strong sunlight the shading is necessarily in proportion to the amount of glass surface exposed. There is no reason, however, why the air-control apparatus described for these chambers would not be equally applicable to chambers constructed with glass walls. The more satisfactory way of increasing the illumination of air-control chambers is by the use of artificial light, with a flowing water screen to absorb the heat from the lamps. The illumination is still far from "normal," however, and chambers may therefore be said to be useful only within certain limits. For our purposes we have found it preferable to rely on natural light, as usually only one to three weeks' exposure of plants in the chambers is found necessary.

The chambers are constructed of three-fourths inch cypress lumber, the bottom and three sides being double walled, with a dead air space and build-

ing paper between. The top and one side are constructed of double-walled, single-strength glass, inserted in door frames on hinges, which are held closed by ordinary window fasteners. The glass side in all chambers faces

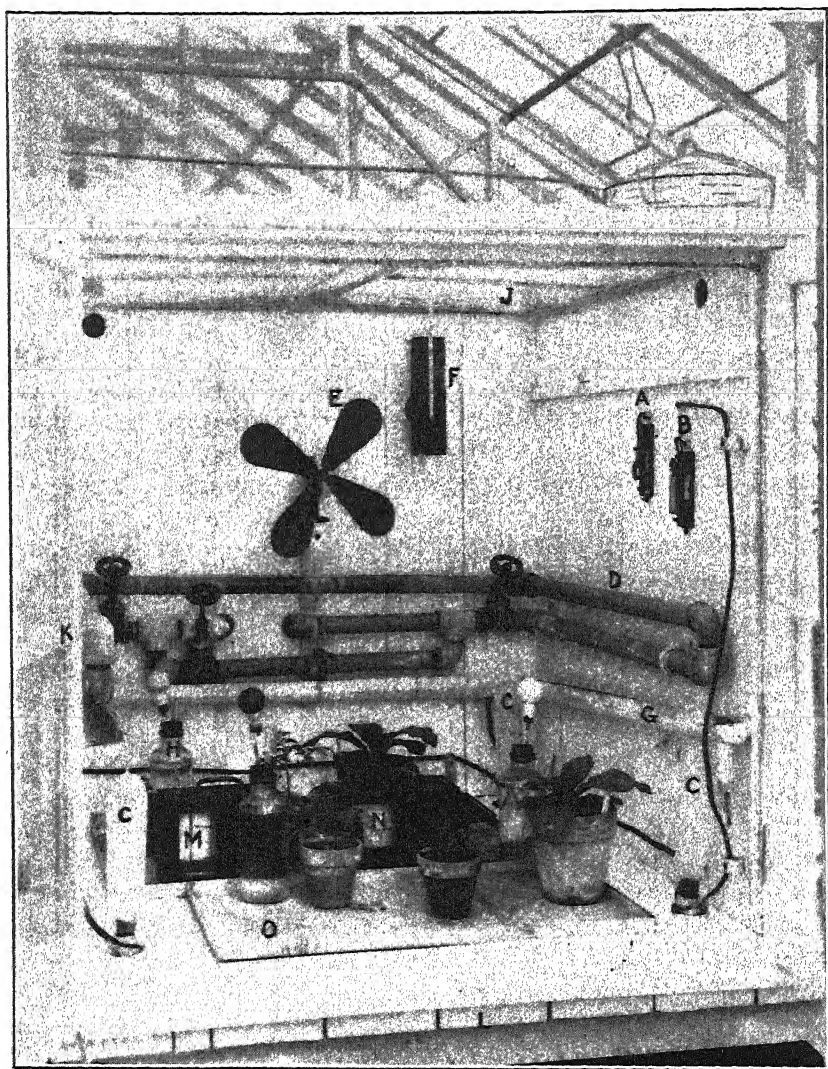


FIG. 1.—Interior view of one of the air-control chambers. A, electric thermostat; B, electric humidostat; C, heaters; D, brine pipes from refrigeration system; E, fan, driven by motor outside chamber; F, inlet for humidified air; G, drain for drip from brine pipes; H, atmometers; J, glass-top in door-frames; K, front glass door; L, plank bench; M, thermograph; N, hygograph; O, drain pan for potted plants.

the south in order to obtain the maximum sunlight. The chambers are also kept painted pure white, which aids in the reflection of light within the chambers. The sides enclosed with glass also have wooden doors which can be attached when light is not desired in the chambers. Each chamber, with its apparatus, rests on a heavily constructed bench about two feet in height. This bench is four by six feet in area, so that four by two feet extend out to one side of the chamber upon which the humidifier "spray-tank" is placed, together with other required apparatus (Fig. 2).

TEMPERATURE REGULATION

The refrigeration system in the chambers has already been referred to. The heaters are of sufficient capacity to maintain a constant temperature against the refrigeration used. The practice is, therefore, to use only the approximate refrigeration surface necessary to hold the temperature down to the required degree during periods of the day when the highest temperatures occur.

Similarly, sufficient heating capacity must be available during periods when the temperature is likely to be at the minimum in the chambers. For our purposes, we have found two or three 250-watt Westinghouse Radiator units to be sufficient (Fig. 1, C). The heating units are placed in an upright position in the corners of the chamber. These heaters are regulated by a Johnson Service Company,² Number 2, electric thermostat (Fig. 1, A), which operates through a relay made by the same company (Fig. 2, F). By means of a transformer on the relay, the 110-volt current is reduced for the thermostat; consequently no batteries for this current are needed, greatly reducing the inconvenience, and adding to the reliability of operation. Aside from cleaning the contact points on the thermostats and relays two or three times a year, this system has required no special attention. The temperature can ordinarily be controlled to within one or two degrees (Fig. 3). During days of strong sunlight, in the spring and summer months, the temperatures are likely to rise unless the glass walls of the chambers are shaded, for which purpose we have used a thin white shade, conveniently hung above the chamber on an ordinary shade-roller so that the shade may be readily raised or lowered as desired.

HUMIDITY REGULATION

The source of the humid air consists of an air current passing through a spray of water held at a temperature a few degrees above that of the chamber to be humidified. The hot-water supply for all chambers flows from the steam-heated and thermostated tank which has been already referred to. The hot and cold water are mixed if necessary to secure the desired temperature. This can be accomplished by merely regulating the amount of

² Milwaukee, Wisconsin.

flow of hot and cold water in the pipe before it reaches the spray-nozzle. For this purpose it is important to have both sources of water on the same pressure line and to use valves which will maintain a constant position when set. The temperature of the water need not be carefully regulated, but for convenience in regulation it is advisable to insert a mercury thermometer beyond the "mixer" or into the spray-tank in a convenient position for reading. If the water used contains some solid material, it is well to pass it through a large screen somewhere in the main water line before it reaches the ordinary mist type of fruit tree spray nozzle which should also contain a screen. The "spray-tank" (Fig. 2, A) used is an upright cylinder, 15 inches in diameter and 5 feet high, in which the spray is confined. The top is a removable cover from which the spray nozzle, which is placed in a vertical position, can be reached for cleaning. At the base of the tank, a drain pipe carries the excess water to the sewer. About one foot above the base of the tank is a four-inch aperture, connected with an air-blower, and between the tank and the blower is a two-way or double-mixing damper enclosed with galvanized iron (Fig. 2, B). Through the humidostat, this damper regulates the passage of the humid air into the chamber. A short three-inch pipe carries the air from the upper end of the spray-tank into the chamber (Fig. 1, F). The blower (Fig. 2, C) used is a Number 0 "Sirroco" motor-driven blower (1/20 H. P.) which operates continually. In our earlier chambers the blower was operated intermittently by the humidostat, but this method was found to be much more injurious to the motors than continual operation. The action of the humidostat is therefore transferred through a special relay (Fig. 2, D) to the electro-magnet or automatic switch (Fig. 2, E) which operates the dampers, turning in or shutting off the humid air as required. The humidostat used is of the electrical contact type, manufactured by the Johnson Service Company, as is the relay, automatic switch, and six-inch double-mixing damper. The dampers can be operated, of course, by any solenoid of sufficient capacity, but we have used the automatic switches, which were originally used on the motors, as these were available, and have found them very satisfactory for this purpose. The covering of the mixing damper and the small levers which connect the automatic switch with the damper are not furnished with the apparatus and must be constructed to fit the particular arrangement used.

With this system of humidity control, the water spray and motors are in continuous operation. The air of the chambers is ordinarily held constant to within about five per cent of relative humidity (Fig. 4) by means of the control apparatus when properly adjusted. There is no circulation of air from the chambers back through the motors and tank, although this could be easily arranged if desired, by connecting the blower and the double mix-

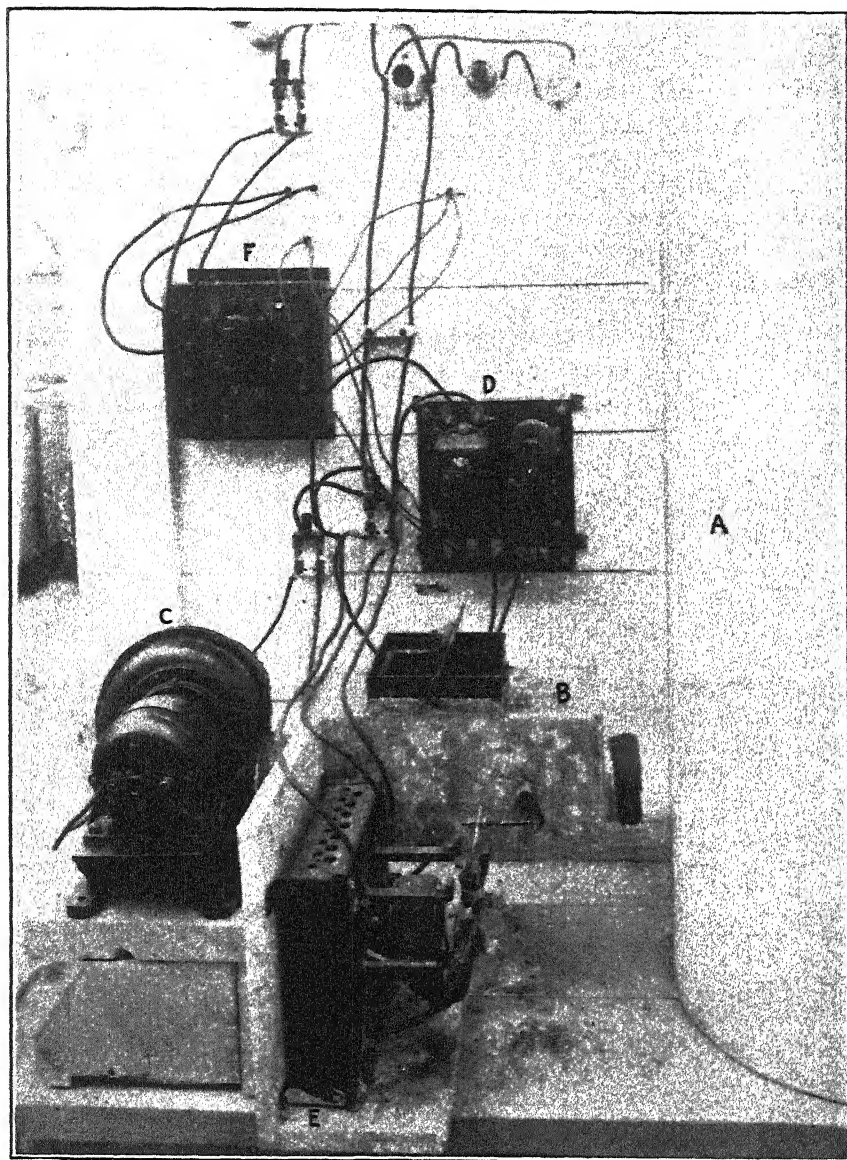


FIG. 2.—Exterior view of one side of air-control chamber where practically all the control equipment is located. A, spray-tank; B, “double-mixing” damper enclosed in galvanized iron box; C, blower; D, relay connected to humidostat; E, automatic switch operating damper; F, relay from thermostat operating heaters. Wiring is according to directions furnished with instruments.

ing damper directly with the chamber. This method of air moisture control is, of course, not applicable for lower relative humidities than will normally occur in the chambers without any humidification. In other words, for drying the air, another system would need to be used. Lower humidities than can be secured in such chambers are not, however, ordinarily required. It seems probable that constant low humidities might be secured by using cold water sprays and rearranging the humidostat connection so as to introduce the cold dry air as the humidity rises. This system has not as yet been given an adequate trial in our chambers.

VENTILATION AND CIRCULATION OF AIR

The ventilation of the chambers is accomplished essentially through the humidifying apparatus. Normally, new air from the greenhouse is blown into the chambers every few minutes by the blower as humidified air. This method may not have any important advantage over recirculating the air in the chambers through the blower for humidification, but has seemed justified in our chambers by the assurance that there was no foul air around the plants. The circulation of the air within each chamber is accomplished by the continuous operation of a 13-inch fan at a low speed (about 200 revolutions a minute) which is sufficient to keep the air in gentle motion (Fig. 1,

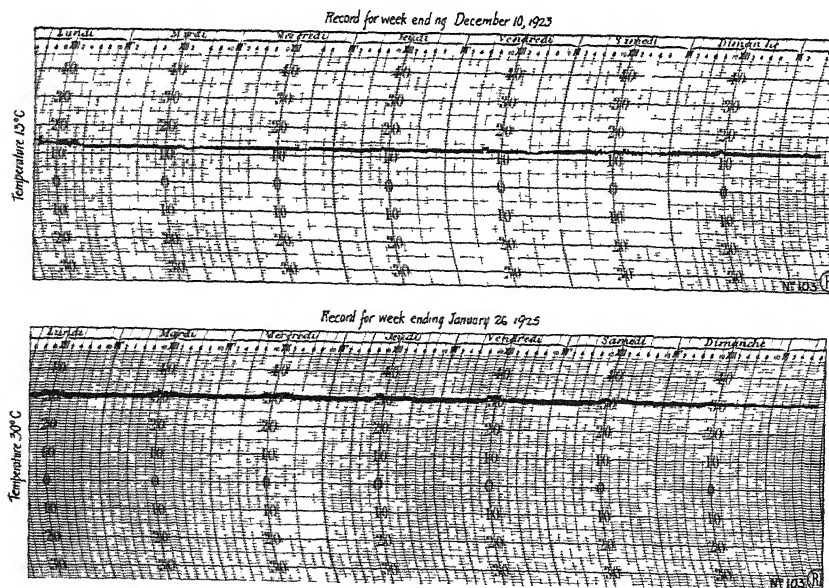


FIG. 3.—Thermograph charts illustrating the approximate constancy of temperature secured within the air control chambers over a period of one week. Note that most of the disturbances occur near midday as a consequence of the influence of direct sunlight.

E). The fan is operated from a small pulley shaft extending through the wall of the chamber, where the shaft is driven by a belt from a small motor resting on a shelf attached to the wall of the chamber. The speed of the fan is therefore determined by the size and arrangement of the pulleys. It seems important to have some such arrangement, because motors operating in the interior of the chamber under conditions of high humidity are likely to be damaged.

RECORDING APPARATUS

It is desirable, if not necessary, to have the proper recording apparatus in each chamber for the records of temperature and humidity. The actual temperature in the chambers is determined from a suspended thermometer, but a thermograph (Fig. 1, M) shows at a glance whether or not the temperature has been constant throughout the week. The hygrograph (Fig. 1, N) is useful, but is not to be regarded as reliable for the actual percentage of relative humidity. For actual relative humidity we have relied upon the use of wet and dry bulb thermometers set in front of a rapid current of air, such as the Friez aspirating psychrometer.

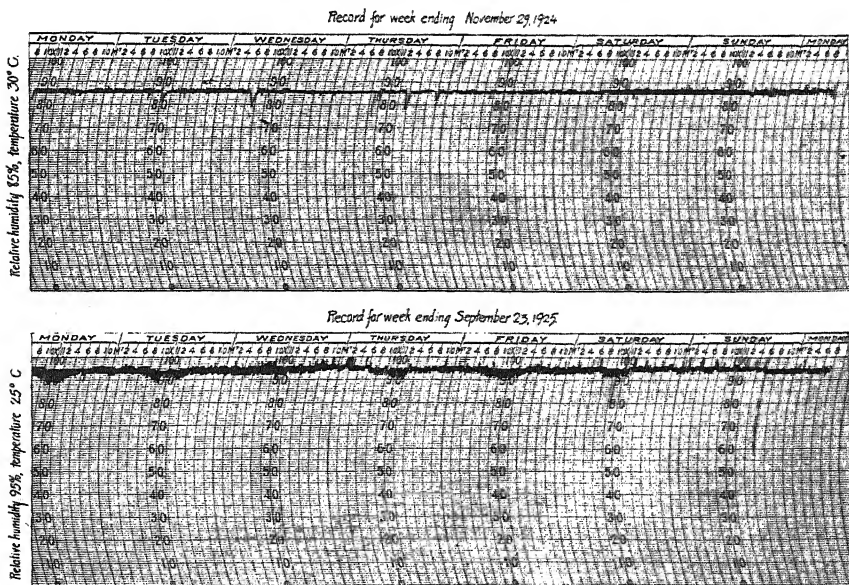


FIG. 4.—Hygrograph charts illustrating the approximate constancy of relative humidity secured within the air-control chambers. Humidity is more difficult to control satisfactorily and unless the instruments are carefully adjusted and kept in good repair much greater variations than shown in these graphs will occur. While the equipment is not infallible, the accuracy obtainable is believed to be sufficient for all ordinary purposes.

The evaporating power of the air naturally varies with the temperature, at any given relative humidity. We have usually planned to regulate the chambers according to the evaporating power of the air rather than according to the relative humidity. For this purpose, we have used three Livingston atmometers in each chamber (Fig. 1, H), the average of which is taken as a measure of the moisture conditions of the air in the chambers.

During one series of experiments, the records show that we have secured an atmosphere in the chambers with an evaporation of only 3.5 cc. a day (approx. 95 per cent rel. hum.) at a temperature of 91° F., as compared with an evaporation of 31.4 cc. a day (approx. 53 per cent rel. hum.) at 75° F. Low humidities with high temperatures and high humidities with low temperatures are, of course, more easily secured than high temperatures with high humidities and low temperatures with low humidities.

The temperatures at which the chambers can be operated are roughly within the range of plant growth. Temperatures as low as 10° C. and as high as 45° C. may be secured. Relative humidities as low as 30 per cent can be secured at the higher temperatures as well as high humidity at most temperatures desired. It is possible, therefore, to maintain approximately the same temperature in all chambers and vary the humidity or *vice versa*.

A great deal of the constancy and reliability of the air-control depends upon the careful adjustment of the instruments and maintaining them in a good condition. Poor electrical contact points account for most of the difficulties of operation. It is preferable to give regular daily attention to the oiling of the motors in order to avoid any danger of overlooking it entirely. Some types of motors are no doubt better than others for continuous operation, but in general they will run continuously for many years with proper attention. While the operation of the chambers should be watched two or three times a day in order to make necessary repairs as soon as possible, they may often operate satisfactorily for weeks with little more attention than the oiling of the motors.

COST OF CONSTRUCTION AND EQUIPMENT

The cost of air-control chambers of the type described in this paper is difficult to present satisfactorily, since much depends upon the equipment already available, such as greenhouse space, steam, running water, drain, electric current, and refrigeration. If part or all of this general equipment must be arranged for, and cannot be used for any other purpose than the operation of the air-control chambers, the total cost of construction will be relatively high. In the case of most institutions planning to erect air-control chambers, such general equipment and conveniences are already available and will not be considered in connection with cost of construction or operation of the air-control chambers. The following is an approximate estimate

of the cost of construction and equipment of one chamber. The carpenter work, material, and painting of the chamber ($4' \times 4' \times 4'$) and bench ($4' \times 6'$) as described may not cost over \$60.00. The electric control equipment, including thermostat, humidostat, with relays for each, one automatic starter switch, and one six-inch double-mixing damper, will amount to about \$125.00. A small motor and shaft with pulleys to operate fan, spray-tank, nozzle, valves, and piping for water and brine and heaters, together with other necessary small equipment, will cost about \$75.00. The hygrograph, thermograph, and Livingston atmometers for one chamber cost approximately \$200.00. Since the latter are not a necessary part of the chambers, and may be used for other purposes as well, they should probably not be charged to the cost of construction of the chambers. Excluding these instruments and assuming the necessary electric current, hot and cold running water, drain and refrigeration are available close at hand, the total cost of constructing one air-control chamber of the type described in this paper will be approximately \$260.00. As three chambers operating simultaneously are usually necessary for satisfactory experimental work, the cost must at once be tripled. Such an estimate is, of course, only useful in giving those who are considering construction of similar apparatus an idea of the funds required.

EXPERIMENTAL RESULTS

It is not the purpose of the present paper to discuss in detail the results secured with these chambers. These have, for the most part, been reported elsewhere. In the first contribution (5) it was shown that the optimal temperature for the development of ordinary tobacco mosaic lies between 28° and 30° C. and that the maximum temperature lies at about 36° – 37° C., at which temperature the symptoms tend to be masked. It was also shown that the optimal temperature for the wildfire disease of tobacco (*Bacterium tabacum*) is relatively high (28° – 32° C.) and that the minimum is below 15° C. and the maximum above 37° C. The optimal temperature for the development of late blight of potatoes (*Phytophthora infestans*), after infection occurred, was found to be relatively high (25° – 32° C.). While the critical temperature for the bacterial wilt of the tomato (*B. solanacearum*) was not determined, one experiment showed, for instance, a much more rapid progress of the disease at 36° C. than at 31° or 28° C.

In a later report (6) the writer showed that the mosaic disease of potatoes (rugose mosaic on Triumph and Green Mountain) is masked at constant temperatures above 24° – 25° C., and that the optimal temperature for the disease is approximately 14° – 18° C. Mention is also made in this paper of the relation of temperature to tomato mosaic, soy bean mosaic, pea-bean mosaic and clover mosaic.

Tompkins (9), working with the same chambers, studied particularly the relation of intermittent exposure of potato mosaic to high temperatures, and arrived at some particularly interesting results from such experiments. In general, it was found that masking and the tendency toward masking could be secured by comparatively short exposures to the higher temperatures of the chambers. This fact is not only significant from a practical point of view, but is of considerable scientific interest as well.

Recently, the writer has described the attenuation of tobacco mosaic (7) secured by exposure of inoculated plants to a constant temperature of 35° C. for ten days in the chambers.

The chambers have been extensively used for studies on the relation of temperature and humidity to the curing of tobacco, the details of which have not yet been published. In a minor way, they have also been used to a considerable extent for other problems, particularly by graduate students. Altogether, the experimental chambers have been very useful, and the results secured have well justified their construction, and have demonstrated the possibilities of further work along such lines either with similar or improved equipment.

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PENICILLIUM INJURY TO CORN SEEDLINGS

HELEN JOHANN

When the seed-corn germination tests were made at the University of Wisconsin in the spring of 1927¹ and the records examined, attention was called to the possibility of *Penicillium* being a factor in the corn-disease problem in Wisconsin. On a number of ears *Penicillium* occurred with other fungi, while on 63 ears only the *Penicillium* was recorded. The average germination of the 63 ears infected with *Penicillium* was 79 per cent, and only 51 per cent of the kernels tested produced healthy seedlings. That is, 28 per cent of the seedlings from the kernels tested showed evident injury apparently from the *Penicillium*. Isolations from the diseased seedlings produced a high percentage of pure cultures of a green *Penicillium*.

The presence of *Penicillium* spp. within seed corn has been reported by Manns and Adams,² who recognized it as a parasite of the storage tissue of the scutellum and in some cases a limiting factor in germination. They, however, did not find it so consistently associated with seed corn that they considered it among the more important parasites.

Koehler³ isolated a species of *Penicillium* from germinating corn kernels and as a result of his inoculation experiments he lists it among the fungi capable of producing scutellum rot. He says, "Scutellum rot of corn is a disease that occurs during the seedling stage of the plant and may be caused by a number of different organisms. On the germinator *Rhizopus* spp. are the most common causal agents." He finds that "Isolations from seedlings grown from susceptible seed in soil at 16° C. showed that species of *Mucor*, *Penicillium*, and *Fusarium* were the predominating organisms associated with scutellum rot."

Preliminary inoculation experiments in the Wisconsin soil-temperature tanks with Holbert's inbred strain B-1-1-3-R-10-1-12 and his cross No. 366,⁴ a first generation hybrid of an inbred Leaming strain and strain A-1-1-2-R-1-1-9, indicate the probability that the *Penicillium* isolated in this laboratory may become a factor in the seedling blight problem under certain

¹ Germinator readings were made by Dr. J. G. Dickson and Mr. P. H. Senn.

² Manns, Thomas F., and J. F. Adams. Parasitic fungi internal of seed corn. Jour. Agr. Res. 23: 495-524. 1923.

³ Koehler, Benjamin. Studies on the scutellum rot diseases of corn. Phytopath. 17: 449-471. 1927.

⁴ Seed of Cross No. 366 was supplied by Dr. J. R. Holbert, Bloomington, Illinois. B-1-1-3-R-10-1-12 was grown at Madison, Wisconsin, by Mr. Paul Hoppe from seed received from Dr. Holbert in 1926.

TABLE 1.—*Reduction in germination and final stand as compared with the control in two lots of corn following seed inoculation with spore suspensions of Fusarium moniliforme, Trichoderma sp., and Penicillium sp.*

Inoculum	Seed ^a	16° C.		20° C.		24° C.		28° C.	
		germina- tion	Reduction in— stand	germina- tion	Reduction in— stand	germina- tion	Reduction in— stand	germina- tion	Reduction in— stand
<i>Fusarium moniliforme</i> {	x366 B 1-1-3-R 10-1-12	per cent 0	per cent 0	per cent 0	per cent 0	per cent 0	per cent 0	per cent 0	per cent 25
		0	6	7	20	13	12	b+6	0
<i>Trichoderma</i> { sp.	x366 B 1-1-3-R 10-1-12	6	0	0	0	0	0	0	0
		c8	8	0	0	0	6	7	8
<i>Penicillium</i> { sp.	x366 B-1-1-3-R-10-1-12	0	19	0	38	0	57	0	62
		e42	58	0	47	6	57	0	64

^a 16 kernels planted in each group unless otherwise stated.

^b + = increase.

^c 12 kernels planted.

conditions. Germination was not seriously affected at any of the temperatures used. Reduction in the final stand occurred in all the tanks. At 28° C. and 24° C. the number of healthy plants remaining at the end of the experiment was less than 50 per cent in both lots of corn. The stand at 20° and 16° C., though not equalling that of the controls, was not reduced to so great an extent as was that at the higher temperatures.

In parallel inoculations in which *Fusarium moniliforme*, *Trichoderma* sp., and *Penicillium* sp. were used, the only consistent and severe injury occurred following the *Penicillium* inoculations (Table 1). This was of special interest in the case of Holbert's cross No. 366, as this vigorous cross had shown resistance to *Gibberella saubinetii*, *Diplodia zeae*, and *Pythium* sp. in a previous inoculation experiment.

The first symptom of invasion by the *Penicillium* was the light yellow-green color of the basal half of the upper leaves. This shaded into a normal green at the tip of the leaf. As the disease progressed, the tips and margins of the leaves became dry. Eventually all the leaves became dry without a striking change of color. Some of the seedlings reached the fourth-leaf or fifth-leaf stage before death occurred. A mass of *Penicillium* usually was plainly visible surrounding the tip of the kernel on the young seedlings which had been inoculated with a spore suspension at time of planting. Infection took place in the embryo region and proceeded up the mesocotyl. In some instances the mesocotyl was rotted at the base; in other cases it appeared to be clean. The fungus was isolated repeatedly from the embryo region and from portions of the mesocotyl which apparently were clean.

Sections, checked by platings of adjacent areas, showed the hyphae to be both inter- and intra-cellular. The mycelium was found to invade both the parenchyma and the vascular elements. Some sections indicated that the cells were killed in advance of the mycelium. This killing may possibly be due to oxalic acid formed by the fungus, as the organism produces numerous oxalate crystals within a short period of time when it is grown on potato dextrose agar.

A culture of the *Penicillium* was submitted to Dr. Charles Thom for determination. He replied that the culture resembled *P. oxalicum* Currie and Thom. According to these authors⁵ *P. oxalicum* has been obtained from corn in Kansas and from moldy corn and cornmeal from Connecticut, Maryland, Virginia, and Illinois. They state that "it also occurs in soil cultures but evidently if it is a soil organism it is so well adapted to grow upon corn that it becomes a very common component of the flora of moldy corn and corn products."

⁵ Currie, James N., and Charles Thom. An oxalic acid producing *Penicillium*. Jour. Biol. Chem. 22: 287-293. 1915.

SUMMARY

1. *Penicillium* spp. have been reported on corn but have not generally been considered among the more important seed-corn parasites.

2. In seed-corn germination tests made at the University of Wisconsin in the spring of 1927, *Penicillium* frequently occurred and apparently injured the seedlings.

3. In inoculation experiments at different temperatures germination was not seriously affected. The greatest reduction of stand occurred at 28° C. and 24° C.

4. In parallel inoculations with *Fusarium moniliforme*, *Trichoderma* sp., and *Penicillium* sp., the only consistent injury occurred following inoculation with *Penicillium*.

5. Some seedlings reached the fourth- or fifth-leaf stage before death occurred. Infection took place in the embryo region and proceeded up the mesocotyl. The hyphae were both inter- and intra-cellular. Both the parenchyma and vascular elements were invaded. The cells were apparently killed in advance of the mycelium.

6. According to Dr. Charles Thom the *Penicillium* isolated from corn and used in the above experiments resembled *P. oxalicum* Currie and Thom.

OFFICE OF CEREAL CROPS AND DISEASES,

BUREAU OF PLANT INDUSTRY,

U. S. DEPARTMENT OF AGRICULTURE, AND

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THE CROWN ELONGATION DISEASE OF THE PEONY

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During late September, 1925, I received from a peony grower in the Middle West a recently dug root of a seedling peony plant about three years old. It was evidently diseased. The most striking symptom of the malady, and about the only one, was a marked elongation of the crowns with small weak buds at the tips. Again early in September, 1927, the same grower sent me another plant affected in the same way. On my request he sent me an additional half dozen or more plants showing the same symptoms. Several of these were of the variety Darkness, which showed the characteristic symptoms in a most striking manner (Fig. 1). A careful examination together with isolations from the roots failed to show the presence of any causal organism. Although I have examined many diseased peony roots in the course of the last 20 years I have never seen this particular disease from any other source so far as I recall. Whether it is contagious or not I can not say. The grower who sent me the specimen however, seems to think that the disease is on the increase in his plantings. He has observed that the disease seems to start on one side of a large clump and gradually involve the entire plant.

It seems desirable at this time to present a photograph and brief description of the symptomatology of this disease. Its most striking feature is a marked elongation of the crowns, the roots remaining apparently normal. These elongated crowns are much more numerous than the crowns on healthy plants, making a witches broom effect (Fig. 1). These numerous elongated crowns appear to originate by excessive bud formation about the base of the originally healthy crowns, which were rotted or partially destroyed in some way and later healed over.

The crowns not only elongate but branch more or less from adventitious buds along their sides. These secondary buds are very numerous. Feeding roots also develop from the elongated crowns as may be seen from figure 1, but they do not appear to be numerous and never thicken up like the storage roots in normal plants. The buds at the tips of these elongated crowns seem to be more advanced in development in the autumn than the buds on normal peony crowns.

In the spring, when the buds from these elongated crowns develop, they send up slender, weak shoots which seldom reach a height of more than 5 or 6 inches, with small dwarfed foliage and no flower buds. The roots apparently live for years but they never produce vigorous, blooming shoots.

A number of these diseased plants have been planted in my garden for further study and observation. Should this prove to be a contagious disease, its prompt eradication from plantations where it occurs is imperative.



FIG. 1 Autumn condition of peony plant affected with crown elongation.

Affected plants never recover, and no attempts to propagate from them should be made. As they apparently never bloom, there would seem to be no reason for retaining them in a plantation.

Plant pathologists are urged to be on the look-out for this disease in peony plantations. The writer would be pleased to receive specimens of this disease from other sources.

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PHYTOPATHOLOGICAL NOTES

Killing of Strawberry Roots. Intermittently since 1922, observations and experiments have been made on root-killing of strawberry plants. These have been discontinued and the following summary of findings is presented.

All the varieties extensively grown in New York State are affected, and no consistent difference in amount of injury has been found.

The injury may appear in localized areas or on scattered plants throughout a planting. Typically the roots are killed from below upward and the plant tends to put out new roots higher up on the crown. In later stages the crown breaks down, beginning usually at the basal end from which the stolon has been detached. The entire plant frequently dies, particularly during a period of drouth at fruiting time or at times of heavy tax on the resources of the plant. Young plants sometimes die during the same season in which they are produced.

Attempts to isolate a pathogenic organism were made by G. R. Hoerner and by myself. Of 188 thrust cultures recorded from 12 sources, 68 plantings were sterile and 37 contained fungi of the *Mucor* type. Fungi of the genus *Cephalosporium* or closely related genera were obtained from at least five sources. In only one instance was *Rhizoctonia* isolated. Cultures from the interior of dying crowns were particularly likely to be sterile.

Nine different fungi and bacteria were used to make inoculations on plants grown in the greenhouse. None of these produced definite symptoms of disease.

Plants grown in pots with soil and with dead plants received from correspondents were as vigorous after several months as were the control plants.

Injured plants tended to recover when potted in greenhouse soil.

A planting of 18 varieties was made in the field at Ithaca, New York. Five of these were obtained from two plantations in which root-killing was abundant. The plants of one variety, Parson's Beauty, came from an area in which more than half the plants had died. Several fungi and soil and plant debris from four other sources were scattered over this planting. During three seasons never more than an occasional plant showed symptoms of root-killing. The variety, Parson's Beauty, was one of the most vigorous and fruitful of the lot. A few rods distant a second planting of the same varieties was made, to serve as a control for the first. Owing to poorer soil and cultural conditions the plants of the second planting were not so well grown or productive as were those of the first.

Strawberry plants are notably sensitive to soil and other environmental conditions. Factors which seem particularly to influence the occurrence of root-killing are: type and exposure of soil, low temperatures, application of lime, mulching practice.

It is concluded that root-killing of strawberries in New York State may result from a number of causes, frequently in combination, and that fungi in most cases at least play a minor rôle.—H. E. THOMAS, Department of Plant Pathology, Cornell University.

Verticillium wilt of heliotrope. In the fall of 1925 conspicuous dead areas were noticed in two large beds of heliotrope on the grounds of the Department of Agriculture in Washington. The plants in these areas were still upright, but the leaves and blossom clusters were black and wilted or shrivelled. Most of the stems were entirely brown, or brown in wide streaks. Examination showed a somewhat discolored vascular region and fungous mycelium in both cortex and wood.

From such stems a *Verticillium* resembling *V. alboatrum* was isolated and used to obtain successful infections on heliotrope in pots in the hot-house. The disease appeared again in 1926 and sporadically in 1927.

The fungus has a wide range of hosts but has not heretofore been reported on heliotrope. Van der Meer,¹ who gives a table of the distribution of *Verticillium* wilt among the dicotyledons, does not cite heliotrope nor any member of the Boraginaceae as a host.—MARY K. BRYAN, Assistant Pathologist, Laboratory of Plant Pathology, Bureau of Plant Industry, U. S. Dept. of Agriculture.

An improvement in the technique for feeding homopterous insects. Since the first report² was made on a method for feeding homopterous insects, which consisted of inclosing a solution in a membranous sack, it has been found that the technique can be advantageously adapted to petri dishes. The new method is to fill one-half of the petri dish with the solution and place a piece of the membrane over the dish, taking care that good contact is made with the surface of the liquid. A rubber band will hold the membrane to the dish. This can then be inclosed in any suitable type of cage, and for nymphs and wingless sucking insects it is in many ways an improvement over the sack which is hung free in the cage. It also permits the use of smaller quantities of solution—as low as two or three cc. if a small petri dish is used.

¹ Van der Meer, J. H. H. *Verticillium wilt of herbaceous and woody plants.* Meded. Landbouwhoogeschool, Wageningen 282. 1925.

² Carter, W. A technic for use with homopterous vectors of plant disease, with special reference to the sugar-beet leaf hopper, *Eutettix tenellus* (Baker). Jour. Agr. Res. 34: 449-453. 1927.

The fishskins described in the original article are expensive and somewhat difficult to obtain, and for that reason a continued search has been made for a suitable substitute. Such a substitute, for most purposes, was found in Baudruche Capping Skins.³ This product comes in sheets averaging 8 or 9 inches in width and from 30 to 36 inches or more in length. Sacks can easily be made from these flat sheets, and for the petri-dish method they are readily cut to fit a petri-dish of any size.

One difficulty has been encountered with this skin. When there is considerable pressure from a quantity of solution inclosed in a sack of this material there tends to be more oozing from the feeding punctures than there would be with the use of the fishskins. There is not this disadvantage, however, in the petri-dish method, as the material is not subjected to any pressure from a large volume of solution. This material is very similar in appearance and texture to the fishskins but perhaps not so strong, and should it prove generally satisfactory it will be very much cheaper and more easily obtained than the original material.—WALTER CARTER, Bureau of Entomology, U. S. Department of Agriculture, Twin Falls, Idaho.

Phytophthora, Pythium, and Pythiacystis species as stock cultures. Supervision of cultures for such an undertaking as the American Type Culture Collection necessitates consideration of artificial culture media, maturity of the organism at the time the culture is placed in storage, humidity conditions in the storage compartment, when possible, and storage temperatures. Observations of stock fungous cultures to be even of suggestive value must be made with a background of continuous experience which is generally not attainable.

It seems possible at present to make note of the reactions of some species of *Phytophthora* and *Pythium* to storage temperatures. This note is based on one series of transfers of stock cultures of these two genera. The data included here were discussed with Dr. Charles Drechsler of the United States Department of Agriculture.

The species on hand were transferred between July 26 and 28 to potato agar. The medium was prepared by adding 500 gms. of pared and finely sliced potato to 1,000 cc. of water, the whole being heated with steam for 45 minutes and then filtered through two or three layers of wet cheesecloth. After filtration 15 gms. of agar was added, and the medium sterilized at 15 lbs. for 15 minutes. Transfers were made by cutting out a piece of the old culture somewhat more than 5 mm. square and placing it at the base of the fresh slant where a small pool of liquid had accumulated. Three sets of *Phytophthora arecae* Pethybridge, *P. cactorum* Schröter (strains 1,

³ The samples used were transparent Baudruche marked 1-A and 1-B and are manufactured by Paul Troeder, Belleville, New Jersey.

2, and 3 of Leonian⁴). *P. capsici* Leonian, *P. colocasia* Raciborski, *P. erythroscptica* Pethyb., *P. faberi* Maub., *P. jagi* Hartig, *P. infestans* DeBary, *P. palmivora* Butler, *P. parasitica* Dastur, *P. parasitica* var. *rhei* Godfrey (strains I through V of Leonian⁴), *P. pini* Leonian, *Pythium debaryanum* Hesse var. *pelargonii* Brauns, *Pythiacystis citrophthora* Sm. and Sm. were transferred in this manner and allowed to develop at room temperature (26 to 32° C.) from two to three weeks. One set was then placed at 0° C., another at 7° C., and the third retained at the room temperature (26 to 32° C.). On retransferring at the end of the two and a half months, viability among the cultures was as follows: of those stored at room temperature only *Pythium infestans* was no longer viable; of the transfers stored at 7° C., *Phytophthora arceae*, *P. colocasiae*, *P. parasitica-rhei* V, and *P. infestans* did not grow on retransfer; of those stored at 0° C., *Phytophthora capsici*, *P. parasitica-rhei* III and *P. pini*, and the species which failed to develop at 7° C., did not grow.

All these cultures or strains were obtained from Leonian by the Collection in 1925, and are discussed in his paper⁴ of that year. Accordingly, they have been maintained in culture tubes for over two years or more by the Collection and others. Recent determination of their ability to produce sound fruiting structures in cornmeal agar does not correlate with their ability to remain viable at the three temperatures under discussion. Accordingly, we may conclude that 0 and 7° C. are unsatisfactory temperatures for the maintenance of these species of *Pythium* and *Phytophthora* under the conditions to which they were submitted and probably for all conditions of artificial maintenance.—MARGARET B. CHURCH, Bureau of Chemistry and Soils, U. S. Department of Agriculture, and MARIO SCANDIFFIO, of the American Type Culture Collection.

⁴ Leonian, Leon H. Physiological studies on the genus *Phytophthora*. Amer. Jour. Bot. 12: 444-498. 1925.

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PRELIMINARY OBSERVATIONS ON SUGAR CANE MYCORRHIZAE AND THEIR RELATIONSHIP TO ROOT DISEASES¹

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INTRODUCTION

Up to the present time the subject of sugar cane mycorrhizae has been considerably overlooked or neglected, not only in relation to the complex and little known group of root diseases, but also as a problem which *per se* should be taken up.²

Only once in the literature of cane diseases is the subject mentioned, when Tryon (23) refers to a work of Treub (22), which unfortunately is unknown to the writer. Tryon, having found a *Pythium* in roots of canes attacked by the "top rot," states that Treub believed this fungus to be a root endophyte and, together with *Heterodera*, the cause of the "sereh." Constantin (8), also referring to Treub, states that the fungus of sugar cane mycorrhizae is known to be a *Pythium*, and in a later publication (9) gives his opinion to the effect that mycorrhizae might possibly be the cause of "sereh."

MORPHOLOGICAL OBSERVATIONS

An examination of longitudinal sections of the rootlets of sugar cane (not the transient rootlets of a cutting) shows that in about 80 per cent of the cases a root endophyte can be noticed in the root system, no matter what the health conditions of the plant may be. It appears that rootlets in their full activity do not show a mycorrhizal development. In the case of initial infection, in spite of the presence of said fungi, only mycelium develops. The development of the mycorrhizae increases as the rootlets become older, reaching a maximum at their maturity, that is, after the second and third month, rarely at the fourth. As a general rule, the mycorrhizal fungus continues to live, associated with other fungi, but as a saprophyte.

¹ The writer is indebted to Professor E. C. Stakman, Editor-in-Chief of PHYTOPATHOLOGY, for his suggestions and criticisms in the final preparation of the paper.

² These preliminary notes comprise the observation and experience accomplished during 18 months. The writer has in preparation a more detailed study on the subject, in which the results of experimental work together with full explanations of some of the topics treated in the present article will be discussed.

Likewise, the extra-radical development of the symbionts, very limited if any during the initial infection, is easily detected in old or decayed roots.

The two types of mycorrhizae, described by Peyronel (20) as "phycomycetous mycelium" and "rhizoctoneous mycelium," may be found as endophytes in sugar cane. It is not easy to differentiate between them if the infection is not in an advanced stage, and especially if the infected rootlets are abnormal either because of the absence of other vegetative organs of the fungi than the mycelium, or more often because of the presence of Hyphomycetes (especially Mucedineous and Tuberculariaceous), of Mucoraceae, and probably of the Hymenomycetoid mycelium which associate with the endophytic fungi in their extra-radical life.

The extra-radical mycelium of the phycomycetous endophyte is formed typically by hyphae of relatively uniform diameter which sometimes enlarge progressively, producing tufts of mycelium, chiefly in the bifurcations of the root. It is very common to find ramified or depressed hyphae, but they are seldom found in groups. When the fungus is cultured in a wet chamber, inducing the saprophytic development of the fungus, the formation of "vesicles" or "sporangia" may be detected, although they are rare in nature. These sporangia are more or less round or ovate and very similar to those previously described by several authors. Up to the present, the author has not been able to see them germinate.

The extra-radical behavior of the phycomycetous endophyte is entirely different from that of the rhizoctoneous endophyte. As a rule the fungus is imperceptible, except where it may be found adhering to the exterior of the rootlets.

The intra-radical development of the phycomycetous mycelium is also slow, but is easily followed in stained material. The mycelium enters the rootlets by penetrating the walls of the epidermal cells, never through the root hairs, and later, in cases of severe infection, spreads across the bark and through the parenchyma. The mycelium is sometimes intercellular, but in most cases intracellular, and generally spreads longitudinally with comparatively few ramifications. The hyphae are of quite uniform size, usually have a thick membrane, although it may sometimes be thin, and contain brilliant, thick protoplasm, as may be found in the extra-radical mycelium. The passage from one layer of cells to another seems to take place normally through the cross walls. Likewise the passage from the exterior to the interior of the roots is accomplished through these cross walls. Frequently it happens that the mycelium expands rapidly and completely fills a cell cavity, and even two or three adjoining cavities; in fact, it takes on a shape very similar to that shown in plates published by Peyronel (20). However, the writer has been unable to observe the repro-

duction of spores recently described by Peyronel (19), and it may be that the expansions are merely expansions of the mycelium.³

Infrequently the phycomycetous endophyte may exist alone in the rootlets, but only during the primary stage of the infection; generally the phycomycetous endophyte is associated with the rhizoctoneous endophyte, although the latter under certain conditions may constitute the only type of mycorrhizal fungus.

The type of fungus constituting the rhizoctoneous endophyte is not different from that studied by the several authors who have discussed the subject of mycorrhizae. Its extra-radical development in tender rootlets of healthy plants is as well pronounced as the intracellular. The hyphae are somewhat irregular, depressed, frequently anastomotic, hyaline to pale brown, and in their intra-radical life generally hyaline to yellow. The formation of Monilia-like chains is generally reduced, but is more abundant in wet soils; and the most favorable conditions for their development are found in a moist chamber at normal temperature. After the infection has started in the piliferous layer of the rootlet, stromata and all the intermediate stages in the development of hyphae are in evidence. The entrance of the endophyte into the rootlets is effected as in the case of the phycomycetous mycelium. The production of pectinase by a species of *Rhizoctonia* (*R. solani* Kühn) has already been demonstrated by Matsumoto (14).

In rootlets of healthy cane the *Rhizoctonia* mycelium does not present special characteristics worthy of mention. In diseased roots both mycelia (which may coexist even in the same cell) show comparatively greater development than in healthy roots, but of the two mycelia the *Rhizoctonia* is always more aggressive in spreading through the tissues. When the hyphae extend almost to the bundles, forming numerous intracellular enlargements, the root is severely affected. When the cortical portion presents longitudinal incisions or depressions it is often possible to disclose the union of mycelia, pseudo stromata, and sclerotia, especially in the central cylinder.

TAXONOMIC POSITION OF THE ENDOPHYTES

Although the writer has not yet been able to determine exactly the taxonomic position of all the endophytes of sugar cane, the subject may be discussed here briefly. Further work is being done.

That the endophyte of the rhizoctoneous type is a *Rhizoctonia* is clear from the appearance of the mycelium, both in the host and in culture. This agrees with the conclusions of several investigators, chiefly as a result of root diseases. Peyronel (20) isolated and cultivated *Rhizoctonia* from

³ The irregular thickenings of the hyphae that sometimes are detected in the interior of cells present characteristics like those described by Bourne (3) in Porto Rico.

three Gramineae, and in these and other plants discovered forms somewhat similar to *Rhizoctonia repens* Bern. and to *R. lanuginosa* Bern. Previously, Janse (12) discovered in *Festuca ovina* an endophyte similar to that examined by Schlicht (21) in *Holcus lanatus*. Recently Jones (13) discovered mycorrhizae in *Panicum capillare*. Matz (15) observed *R. grisea* (Stevenson) Matz, *R. pallida* Matz, and *R. ferruginea* Matz in roots of sugar cane. Peltier (17) made inoculation experiments with a species of *Rhizoctonia* isolated by Edgerton in 1912.

In the present experiments the author was able to isolate two different species of *Rhizoctonia*, provisionally designated as strains A and B. Strain A is undoubtedly the most common in the canes under observation: out of 16 successful isolations, following the well-known practice of Burgeff (6) and Peyronel (20), from healthy canes of different varieties collected from different places, strain A appeared in 14 cases, strain B in 2. As already mentioned, however, the writer did not make a comparative study to ascertain the exact identity of the two strains.

The phycomycetous endophyte of sugar cane is possibly not a *Pythium*, although a *Pythium* has sometimes been isolated. On different occasions a *Pythium* has been reported as a parasite on the root of the sugar cane. The first observation was made by Treub (22) and the next by Wakker (24) in Java. In Porto Rico, Matz (15) isolated the previously mentioned *Rhizoctonias* and a *Pythium* which he believed to be *P. de baryanum*. Carpenter (7) in Hawaii believed a *Pythium* to be the agent causing the root disease (especially the "Lahaine disease") of cane and other plants. Bourne (3) studied a *Pythium* found in roots of sugar cane in Porto Rico, the *P. de baryanum* or *Nematosporangium aphanidermatum* (Ed.) Fitzpatr. (*Pythium butleri* Subramanan).

The literature on the endophytic characters of the *Pythium* is very limited. In addition to the observations of Treub (22) already mentioned, Bruchman (4) described an endophytic *Pythium* in the prothallium of *Lycopodium*.

In only 2 of our 16 tests with diseased roots of sugar cane were we able to isolate a *Pythium*. From its morphological character it might possibly be identified as *P. debaryanum*. Out of 18 tests with young roots of healthy cane this *Pythium* was discovered once when the phycomycetous endophyte was also present. On the other hand, in 3 of 10 experiments the writer was able to isolate the same fungus from roots so entirely invaded by various saprophytes that it was almost impossible to identify the phycomycetous endophyte with the aid of the microscope. Could the *Pythium* itself, which under different environmental conditions and at certain stages of growth of the rootlets is a parasite on sugar cane, be the phycomycetous

endophyte? It is impossible to answer this question without further study. From our experiments we are inclined to believe that an unknown phycomycetous endophyte and sometimes a parasitic *Pythium* may be discovered on roots of the sugar cane; the former can not be cultured artificially while the latter can be. This explains why the attempts to isolate the phycomycetous endophyte have resulted in the eventual discovery and culture of a *Pythium*, and that Treub, at a time when knowledge on the matter was not so advanced, could identify a parasitic *Pythium* with a phycomycetous endophyte.

Incidentally we might note that both *Panicum barbinode* and *P. maximum* have mycorrhizae, although the mycorrhization may not be constant and is often very limited; in the same plot of land may be found plants affected with endophytes, apparently of phycomycetous and rhizoctoneous types, and plants free from them. The presence of a *Rhizoctonia* different from strains A and B was demonstrated by culture experiments.

According to the investigations of Wakker (24), Tryon (23), and others, the rootlets of the diseased cane show a breaking-down of the cortical tissue, often during the second, third, or fourth months. The cortex falls off and the rootlets finally decay. Tryon, although not finding the situation abnormal, states that the entrance of saprophytes through the longitudinal ruptures of the bark fosters the decay of the rootlets.

A thorough study of this phenomenon shows that as a general rule the rhizoctoneous type of mycelium is present. The fungus can be grown as a saprophyte in a moist chamber. The presence of the mycelium can be detected if the rootlet is examined when it has become a pale brown color, before the falling off of the parenchyma. At a later stage the rootlet is invaded by other saprophytes which usually tend to mislead the observer. However, we are not in a position to state whether the functional inertia and the decay of the rootlets is caused by *Rhizoctonia*, as we have not been able to demonstrate it by actual experimental work. If the parasite is the rhizoctoneous mycelium, it is indeed a weak parasite, for, while the infection is initiated when the rootlets begin their activity (when the parasite is apparently harmless), the rootlets begin to show symptoms of weakness and decay after the second, third, or fourth month. If the rootlets are not apparently injured, they derive no advantage from the endophyte, and it is absolutely necessary to make experiments to define the case. Our experiments are not complete; therefore no conclusive evidence has been obtained.

Petri (18) and Peyronel (20) are of the opinion that the parasitism of the mycorrhizal fungi is a "refined" parasitism in which the fungus does not kill the host plant but stimulates the nutritive process. In an indirect way this may be possible in sugar cane, if we consider that the stimulus

would not be shown in the infected roots alone, but in the whole plant, which requires a superproduction of roots.⁴ To find some reasonable explanation for this hypothesis, it would be necessary to do some experimental work with cane both with and without mycorrhizae, the experiments to continue until the canes mature. For the present, we conclude that the mycorrhizal fungi are apparently harmless to plants at an early age and harmful at a later stage, developing into a form of parasitism.

The tests with *Rhizoctonia*, especially with strain A, can easily be made under favorable conditions, as may be noted from the inoculation experiments made by Matz (15), Bourne (2), Peltier (17), *et al.* The difficulty will be to keep the soil aseptic without disturbing the transpiration of the plant which is correlated with the absorbing activity of the roots, principally when the cane reaches a height of 30 to 40 centimeters. The strain A of *Rhizoctonia* does well in sterilized soil rich in humus with a mixture of leaf mold and decayed stems. A great number of the rootlets are entirely invaded by the fungus as soon as they have attained full development and the appearance already mentioned. The fungus continues its development saprophytically in the soil, especially if it is kept wet at all times. The check plants in sterilized soil, without endophytic organism, grow just as well as the plants with the fungi during the limited duration of the experiments. As soon as conditions are rendered unfavorable for the development of the plants, chiefly through inefficient transpiration, the rootlets of plants grown in the presence of *Rhizoctonia* show an altered condition very similar to the normal retardation already described. The rootlet turns reddish and rapidly darkens at the tip; then the bark decays. Not only is this phenomenon constant and the cause of abnormal conditions in the plants, either owing to excess or lack of humidity; but it is also the first sign of disease in the cane, appearing when the above-ground portion of the cane does not yet indicate any abnormal state of the roots—even when the environmental conditions are unfavorable—although the most delicate plants may appear slightly weakened.

Experiments with plants grown under the same conditions of environment showed that the root system appeared, as a general rule, to be better developed in plants with endophytes than in plants free from mycorrhizae, either with more abundant ramifications or longer roots, but it is not possible to make a definite statement in this connection because the plants had not reached full maturity.

The test in which the phycomycetous endophyte was present was made in pots of sterilized soil in which pieces of roots had been allowed to rot

⁴ A normal superproduction if we consider the endophitism and its effects as being a normal phenomenon.

and on which only the phycomycetous mycelium was known to exist. The mycelium of the endophyte could not be discovered in the roots of the canes planted. Cuttings previously disinfected externally were inoculated with a culture of a *Pythium*, under conditions which were not definitely controlled, but in which humidity played an important part. The fungus rapidly invaded the rootlets, even though they had not attained full development, and gradually spread through the cortical parenchyma. This fungus also produced, more or less uniformly, a diffused browning of the root and slow death, without generally producing decay; a phenomenon somewhat similar to mummification. Sometimes the rootlets, although infected, were not severely injured. The *Pythium* infection is probably less common than that of the *Rhizoctonia* strain A. The *Pythium* mycelium, chiefly at the time of initial infection, may easily be confused with the phycomycetous endophyte.

The plants artificially inoculated with the *Pythium* and those naturally infected with the phycomycetous endophyte may also be infected by the rhizoctoneous fungus by transplanting affected plants to pots of soil inoculated with *Rhizoctonia*. The infection of the roots inoculated with the *Pythium* takes place rapidly while it is slow in the case of the phycomycetous endophyte. The new mycelium develops profusely and after a few days the roots decay. However, in cuttings of cane inoculated with a culture of *Pythium* and grown in pots of soil inoculated with the *Rhizoctonia* strain A, the first fungus generally penetrates some rootlets, and on their complete development a severe infection of *Rhizoctonia* follows, either in the roots invaded by the *Pythium*, which then decay, or in healthy roots; and when the vitality of the plant declines, the endophyte apparently develops into a parasite. The intra-radical development of the *Rhizoctonia*, unlike that of *Pythium*, seems to take advantage of deficient humidity.

It is not easy to arrive at definite conclusions from our experiments; sometimes results are contradictory and incomplete. The rhizoctoneous mycelium in roots has the nature of an endophyte, and when the plant has been grown under poor conditions, or when the infection has had two or three months' duration, the fungus may turn into a parasite. The phycomycetous endophyte in rootlets does not develop fully and under ordinary conditions does not behave parasitically, although its presence fosters the development of the *Rhizoctonia* which is more virulent. The *Pythium* seems to be a true parasite which requires special environmental conditions for its development and action. In normal cases, and assuming that the three fungi exist in the soil, the *Pythium* would determine, under certain conditions, the outbreak of root diseases, probably being assisted in this by the contemporaneous development of the *Rhizoctonia*; while the latter, a

regular endophyte, might possibly be blamed for the general weakness of the roots, without causing a disease. The resistance of the cane to *Pythium* has not been clearly determined. In the case of *Rhizoctonia* the result was the formation of new roots, which is possibly an indirect stimulation caused by the same endophyte. The behavior of the phycomycetous endophyte, if this, as the writer believes, is different from the *Pythium* (in spite of some contradictory facts), is rather obscure. The fact that it is never found alone, but always in association with the *Rhizoctonia* or *Pythium* or with both, and the more luxuriant development and aggressive nature of the *Rhizoctonia*, account for the impossibility of distinguishing between the factors. Such being the case, only experiments under special control conditions may decide this question.

RELATIONS OF ENVIRONMENTAL CONDITIONS

Under normal conditions, the reaction of the cane against the infection of endophytes, as already explained, consists chiefly in the formation of new roots. An equilibrium is established between the physiological inactivity of the roots and the formation of new and active roots, that is, an equilibrium between the parasitic activity of the endophytes and the formative stimulus of the plant, and it is then that the influence of the environmental conditions need to be determined.⁵

As a direct result of what has been stated, any condition of the unfavorable environment which interferes with the equilibrium between the loss of activity of the roots, due to the parasitism of the endophytes and the formation of new roots, may be considered as the indirect cause of root disease.

It might be possible that favorable environmental conditions have little effect on the two kinds of fungi, assuming that even under unfavorable conditions they exist in the soil of sugar cane plantations, where organic matter is generally found, and that such conditions would tend to facilitate the saprophytic development of the fungi; but this possibility can not be accepted until the possibility of the virulence of the fungi is demonstrated or their special adaptation to the endophytic function under special conditions. Any condition which prevents the plant forming new roots and interferes with its metabolic activities favors the development of root disease. The plant dies when it is no longer able to compensate for the loss of affected rootlets. On the other hand, the conditions which favor the development and vegetative activity of the cane would likewise be unfavorable for the development of the root disease.

⁵ In speaking of the parasitic activity of the endophytes, the writer has considered the joint action of the two types.

Effect of soil moisture

To a certain extent the ideas expressed above were verified by experimental work. Several cuttings were inoculated with *Rhizoctonia* strain A and the *Pythium* sp., and later planted in boxes, with the sole aim in mind of ascertaining the existence of the two fungi. In all the experiments a number of check plants were used. The plants were allowed to grow to a height of 20 centimeters under normal conditions; then a number of plants were deprived of water, some were watered abundantly every day, another lot of plants was transplanted to soil especially rich in organic matter (manure and leaf mold), a fourth set was transplanted to soil where the same fertilizers were applied moderately, and still another group was transplanted to soil where a moderate application of a mixture of phosphorus and nitrogen was administered. The check plants were growing without fertilizer, but were regularly watered.

Periodical observation of the roots under the microscope indicated that in normal environmental conditions the affected plants grew less than the check plants and the root system appeared slightly infected: about 50 per cent of the rootlets appeared infected by both fungi, with constant renewal of infection. Excessive water applied to the underground portion of the plants resulted in the death of 90 per cent during the period varying from 40 days to 3 months; practically all the rootlets were contaminated by both *Pythium* and *Rhizoctonia*, and the growth of new roots was less than in the check plants. All the unwatered plants died, showing a comparatively small percentage of rootlets invaded by the *Pythium* and the majority infected by the *Rhizoctonia*, the formation of new roots being next to impossible. The infection by *Rhizoctonia* alone, while not reducing to a minimum the vitality of the check plants, did not apparently injure the plants cultivated under excessive humidity during the first three months; after this period the plants showed a severe chlorosis, and in about four and a half months 50 per cent of them died. The endophyte ramified through a great portion of the rootlets; and the formation of new roots was at first regular and appeared to continue without difficulty. Under almost drought conditions the development of the plants was very limited and the death rate high (about 80 per cent); mycorrhization was heavy (not total), and the emission of new roots, particularly after the third month, very rare.

Effect of soil nutrients

The influence of fertilizers was not clearly shown. A probable increase of mycorrhizae was noted in plants grown in soil rich in organic matter, due to the rhizoctoneous endophyte, but there was apparently no change in the parasitism of the *Pythium*. The effect of fertilizing with mineral

matter was more definite: a vigorous general development of the cane, including the root system, was evident; there was also a comparatively smaller number of rootlets infected with the rhizoctoneous endophyte than in the check plants, and a very limited infection by the other fungus.

Effect of climatic and edaphic conditions

Several authors who have previously studied the root disease believed it to be due to, or favored by, different and contrary environmental conditions, as, for instance, excessive moisture or severe drought, or differences in physical or chemical composition of the soil, etc. These observations, apparently contradictory, may well be explained or accounted for by examining the relationship between plants and their environment and their relative quantitative values.

The excessive moisture of the soil, while not favorable to root activity nor interfering with the formation of new roots, favors the saprophytic development of fungi in the soil.⁶ On the other hand, severe drought might constitute a favorable condition for root diseases, the development of the roots being very slow, as explained by Petri (18) in the study of the mycorrhizae of the olive tree. New roots are formed with some difficulty; consequently the plant weakens and can not offer high resistance. The fact that under such conditions the saprophytic life of the fungi is made difficult does not suffice to balance the slow and inefficient development of the root which appears to be the principal factor in resistance to rot.

The lack of soil cultivation which is noted in many sugar cane plantations contributes to the formation of a network of saprophytes, among which species of *Rhizoctonia* are very frequent. The lack of aeration in the soil tends to check the functional activities of the root. The organic matter in the soil affords a good substratum for the endophytic fungi, and organic matter together with green manure may facilitate the development of the root disease when the plants are not vigorous enough to counterbalance the action of the fungi.

The evil influence is especially felt when combined with other unfavorable conditions, such as a marked alkalinity or acidity in the soil. Alkaline soil constitutes the best medium for the saprophytic development of these fungi, apart from the fact that some varieties of cane do not thrive well in this type of soil. Although not having observed canes planted on acid soils, the writer is of the opinion that canes are predisposed toward root diseases

⁶ The development of fungi, chiefly the *Rhizoctonias*, to a few centimeters depth in the vegetable layer of the soil is under such conditions extremely luxuriant. This fact was noted by several investigators, among whom is Peyronel (20); a similar observation has been made regarding the *Phycomycetes* by De Bruyn (5), Dufrénoy (10), *et al.*

because of the unfavorable influences of acid soils on the development of the root system.

Heavy soil seems to favor the development of roots if the amount of clay is not excessive. While a compact clay ground is unfavorable to root development, a soft clay loam is favorable to the plant, as well as a sandy loam that is rich in assimilable substances. The two types of soil frequently influence root development: on light soils roots grow deeper and sometimes the rootlets are apical; on heavy soils numerous lateral roots are formed which do not penetrate so deep into the soil. But we are unable to ascertain what relation exists between these two types of root development and the mycorrhization and root diseases.

As stated by Earle (11), it is possible that some varieties of cane under certain conditions show differences in susceptibility to the root disease, but there is no conclusive evidence. The writer has not carried out experiments with different varieties of cane grown under different environmental conditions.

PREVENTION OF ROOT DISEASES

The best and perhaps the only way to prevent root diseases, as previously explained, is to grow the plants under the conditions which are best adapted for their growth and vitality; consequently any undesirable agent should be eliminated and proper cultivation practiced whenever required.

Experiments were made to prevent infection by endophytic fungi and *Pythium* by means of a partial sterilization of the soil, using copper sulphate and sodium arsenate. Partial sterilization of the soil as a prevention against diseases and plant parasites was investigated by Miège (16) and by Bezzsonoff (1). Although these experiments are still being conducted, it is anticipated that little will be accomplished along this line. The *Pythium* is easily affected by the copper sulphate and also by the arsenic compound. The *Rhizoctonia* strain A shows little reaction to either compound with the dose applied. The effect of sterilization is of short duration and the benefits derived therefrom very limited. Sterilization might be of use before the planting operation but not during the growth of the plants. The direct control of the disease in the soil is impossible, and efforts should be directed toward prevention.

SUMMARY

1. These preliminary observations embody the results of experiments and investigations on the mycorrhizae of sugar cane and their relation to the root disease previously considered to be caused by Hymeniales.

2. Diseased or healthy rootlets of cane may be invaded by both a phycomycetous endophyte and a rhizoctoneous endophyte, or by the latter alone.

A *Pythium* sp. probably different from the phycomycetous endophyte may be found associated with it.

3. There are two strains of the rhizoctoneous endophyte, A and B.

4. The phycomycetous endophyte can not be cultured artificially, and therefore its systematic position can not be well defined; the rhizoctoneous endophyte was cultivated.

5. It may be possible that the "normal retardation and death of the rootlets of the sugar cane" in relation to the presence of the rhizoctoneous endophyte may at first be either neutral, or indirectly a stimulus to the formation of new rootlets, and later take on a parasitic nature. The behavior of the phycomycetous endophyte when alone is unknown. The *Pythium* appears to be a parasite at all times.

6. The death of rootlets previous to the normal retardation is undoubtedly related to the action of the endophytes, and the development of the root diseases results from the loss of equilibrium between the dying rootlets and new rootlets being formed.

7. Consequently any cause which hinders the rapid formation of new rootlets favors the outbreak of root diseases; excessive moisture, severe drought, high acidity or alkalinity in the soil, lack of cultivation, special physical or chemical composition of soils, etc., may be of decided importance.

8. Means of control against this type of root diseases do not show great possibilities. Growing the cane under the best conditions for development and vitality would tend to maintain the equilibrium between the functional inactivity of diseased rootlets and the formation of new ones.

9. Further research on the subject is being done by the writer, and the results of succeeding investigations will be published at a later date.

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PREDISPOSITION OF SUGAR-BEETS TO LATE ROOTROT¹

GEORGE STEWART² AND D. W. PITTMAN³

During the last few years sugar-beets in the Rocky Mountain region have been subjected to a series of disastrous epidemics. Of these, much the most important, if not the only ones of serious consequence, are curly-top and late rootrot.

The sugar-beet leafhopper (*Eutettix tenellus*), or white fly, as it is locally designated, probably transmits a virus. Because of the characteristic curled and dwarfed leaf the disease is known as curly-top. On account of the generally unsuccessful attempts either to control or to prevent curly-top, and on account of reported observation of some resistance in certain strains,⁴ there is a general feeling that plant-breeding is the one really promising method of attack yet to be tried for that trouble. The genetic search for a strain of sugar-beet resistant to the virus transmitted by the leafhopper is likely to occupy a considerable part of the future research program for sugar-beets.

Many field men of sugar companies say that proper maintenance of soil productivity and of optimum moisture conditions for rapid growth influence, in a marked degree, the injury due to curly-top. Careful observation by various workers of the Utah Agricultural Experiment Station, as well as by other scientific workers, show that this position is probably untenable. In the last ten years there have been five epidemics in the Utah-Idaho region—in 1917, 1919, 1921, 1924, and 1926. In addition, there was much loss in 1923. The trouble is rather common throughout the Rocky Mountain region. A similar and perhaps identical condition has caused much trouble in Michigan.

In 1926 curly-top was especially abundant. To it was popularly attributed all of the injury, not only that actually caused by curly-top, but also that caused by late rootrot, which in many parts of Utah and south-eastern Idaho probably accounted for considerably more than half the trouble.

¹ Contribution from the Department of Agronomy, Utah Agricultural Experiment Station. Approved for publication by Director, November 12, 1927.

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⁴ Carsner, E. Resistance of sugar-beets to curly-top. U. S. Dept. Agr. Dept. Circ. 383. 1926.

Since many farmers, and even many fieldmen of the sugar corporations, have failed to distinguish between late rootrot and curly-top which occurred simultaneously in the same field, it is not surprising that they interpreted the effect of good soil treatments on rootrot as an effect on curly-top. In 1926 there was only about 1 per cent loss due to curly-top on all of the sugar-beet plats of the Central Experimental Farm, North Logan. The fact that production in some plats was extremely high and in others extremely low affected the percentage of infection not at all.

In the case of rootrot, however, the predisposing effects of low soil productivity and of unfavorable soil moisture are clear-cut.

This paper has to do chiefly with the disease herein called late rootrot for the want of a better name. Pathologists who studied the epidemic in 1921 reported that the rot begins in June or July at the tips of the rootlets, which, as the rot progresses, begin to turn brown and become dry much as if scorched by a fire, the outer leaves being first affected (Fig. 1). The tops then die and become entirely prostrate. The tap root of the affected beet rots rapidly, usually a brownish-gray, rather dry mass in some cases and in

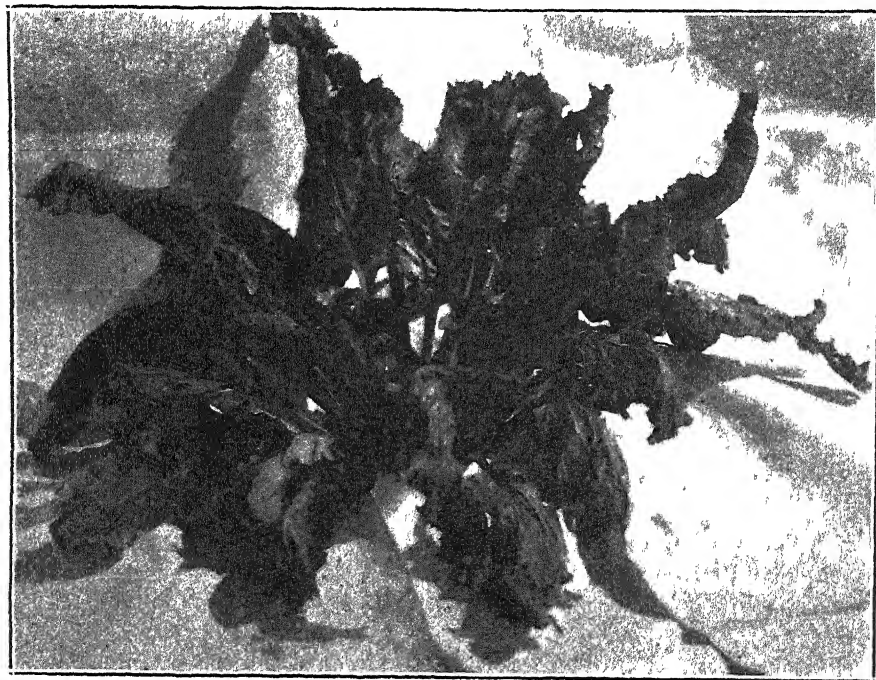


FIG. 1. General appearance of a sugar beet plant in the early stages of late rootrot. The top takes on a burned appearance which spreads rapidly in epidemic years.

others a soft, dark-colored, mushy mass. During the later stages *Phoma betae* Frank and various *Fusaria* have been isolated,⁵ sometimes singly and sometimes conjointly. Other organisms occur also. It was thought likely by pathologists that the initial trouble came earlier in the season and was probably due to some other cause, the *Phoma* and the *Fusarium* coming in as secondary invaders. Whatever the original cause may be, the decrease in vigor of the plant resultant on unfavorable moisture and fertility conditions, is closely associated.

In the epidemic year of 1924 the fields were rather generally damaged. In 1925 all beets on land that was cared for even reasonably well continued in a fresh, vigorous condition up to the harvest. In 1926, following a winter of little rainfall, serious trouble was clearly predicable in April, after a survey of the snow on the watersheds had been made. Injury began almost immediately. Seedbeds were dry; and where irrigation water was not promptly applied, and in many cases where it was, the leaves appeared unthrifty. More than four-fifths of the fields were affected: the leaves began to turn brown in early June; in two weeks, plants were dying in spots which increased rapidly in size. By August, many fields were nearly 100 per cent brown and dying, the trouble being greatly accentuated by a season of drouth in which the irrigation water available was only about 50 per cent of the normal amount.

With fully 80 per cent of the fields attacked, and the damage ranging from a trace to 100 per cent, the total loss mounted rapidly. The average yield for Cache Valley, Utah, in 1925, was 16¼ tons an acre; in 1924, only 7 tons; and in 1926 only 9 tons. The normal is about 12 tons. Thus the year 1925 was very favorable and 1924 and 1926 extremely unfavorable. The returns from sugar-beets in Cache Valley for 1926 alone were about a half million dollars below normal. In Utah and Idaho, it could not have been far from 5 or 6 millions below par. The 1924 loss was even greater.⁶

Part of the loss was due to curly-top, principally so in the southern extension of the sugar-beet area of Utah and in the western extension in Idaho. In Cache Valley, Utah, however, it was predominantly due to late rootrot.

A field survey in 1921 by the Plant Pathology Department, and also one in 1921 and 1923 by the Agronomy Department, of the Utah Agricultural Experiment Station, showed the trouble to be related to soil moisture and to soil productivity. Results of the Plant Pathology Department survey

⁵ Thanks are due to Dr. B. L. Richards, Plant Pathologist of the Utah Agricultural Experiment Station, and to Dr. C. M. Tompkins, of the Office of Sugar Plant Investigations, U. S. D. A., for identifications of rot organisms in late-season stages.

⁶ Thanks for these figures are due to the agriculturists of the Cache Valley unit of the Amalgamated Sugar Company, who kindly made them available.

TABLE 1.—*Soil treatments and acre-yields of sugar-beets on plats of low, intermediate, and high productivity in the two years of serious late rootrot injury—1923 and 1926*

Plat numbers	Soil treatment	Acre-yield in tons	
		1923	1926
<i>Low productivity plats</i>			
5G, 6G	Four-year rotation, clover, potatoes, beets, wheat (no manure)	6.5	0.7
13G, 17G	Alternate wheat-beets, no manure	2.6	0.9
16G, 20G	Alternate wheat-beets, ½ green manure ^a	3.1	1.7
35G	Continuous beets, no manure (check)	8.5	4.1
34G, 38G	Alternate peas-beets, no manure	9.6	4.7
39G	Continuous beets, no manure (check)	9.0	5.2
36G, 40G	Alternate peas-beets, green manure	6.7	7.4
46G	Continuous beets, no manure (check)	7.1	9.1
47G	Alternate corn-beets, no manure	2.4	2.6
51G, 54G	Six-year rotation wheat-potatoes, 2 yrs. peas, 2 yrs. beets (1st yr.) no manure	3.1	1.6
52G, 55G	do do	3.1	2.6
9G, 10G	Four-year rotation, clover, potatoes, beets, wheat, ¼ manure ^a	6.4	7.6
	Average	5.1	3.6
<i>Intermediate productivity plats</i>			
14G, 18G	Alternate wheat-beets, ½ manure ^a	10.5	15.0
15G, 19G	Alternate wheat-beets, ¼ manure ^a	10.0	11.5
86G, 83G	Ten-year rotation, alfalfa, potatoes, beets, peas, wheat, 3/10 manure ^a	16.0	13.0
88G, 85G	do do	9.6	18.8
96G, 93G	do do	19.6	15.8
98G, 95G	do do	15.7	25.7
	Average	10.9	16.6

TABLE 1.—Continued

Plot numbers	Soil treatment	Acre-yield in tons	
		1923	1926
	<i>High productivity plots</i>		
22G, 25G	Seven-year rotation, alfalfa, oats, beets, 2/7 manure ^a	21.8	20.2
23G, 26G	do	13.6	18.4
28G	Continuous beets—10 tons manure annually	27.3	29.0
29G	do —15 do	23.7	25.4
30G	do —5 do	20.6	23.2
30G	do —30 do	26.1	28.3
44G	do —10 do	22.2	21.3
45G	do —20 do	11.1	23.6
48G	Alternate peas-beets, 1/2 manure ^a	12.7	20.0
37G, 49G	Continuous beets, (20 tons manure, alternate years)		
50G	Ten-year rotation, alfalfa, beets, 3/10 manure ^a	19.0	21.9
46F, 43F	do	24.9	25.9
48F, 45F	do	21.4	24.4
56F, 53F	do	24.8	23.1
58F, 55F	do	15.0	24.4
66F, 63F	do	21.3	20.4
68F, 65F	do	19.8	18.6
76F, 73F	do	20.8	24.6
78F, 75F	do		
	Average.....	21.6	24.6

^a The lower figure of the fraction occurring in front of the word "manure" indicates the length of the crop rotation used on the plot series, and the upper figure of the fraction the number of years in the rotation in which manure was applied at the rate of 10 tons to the acre. Thus, "2/7 manure" means that in a 7-year rotation manure was applied twice, usually on sugar-beets.

were published locally in 1922;⁷ those of the Agronomy Department survey form part of this article, having been withheld until more experimental plat data became available. These were obtained in 1923 and 1926. The data of 1924 were not sufficiently detailed to permit a close study.

EFFECT OF SOIL PRODUCTIVITY

For a number of years sugar-beets have been grown under various rotation and manuring treatments at the Central Experimental Farm, North Logan, of the Utah Experiment Station. In table 1 are given the yields during two epidemic years, 1923 and 1926. These are grouped into three classes according to the productivity of the soil in non-epidemic years, as follows: (1) low productivity, (2) intermediate productivity, (3) high productivity. Many of the treatments began as late as 1922; therefore the soils had not reached their true state of productivity by 1923. Some of these treatments, which began in 1922 on depleted land, after sufficient time will bring the soil into a condition of high productivity.

Since, however, there has not yet been sufficient time to accomplish this, the plats receiving such treatments are ranked as of low or of medium productivity. These plats in the lower section of the table which have "F" as part of their number designation, even though treatments began only in 1922, are classified as being in a condition of high productivity because the land had been manured for 20 years previously with about 10 tons an acre of farm manure annually.

The continuous beet plats that were manured received an application every year. As the effort here is to study the effect of productivity on the prevalence of rootrot disease rather than to discover the particular treatments that bring about conditions of varying productivity, this latter question is not here considered.

Table 2 shows for 1926 the number of beets harvested, the number of diseased beets about three weeks before harvest, and the percentage of beets diseased with rootrot when the number harvested on each plat is regarded as 100 per cent.

On the plats of low productivity the percentage of beets affected varied from more than 71 to less than 9 per cent. The mean was 29 per cent. For the plats of intermediate productivity, the range was from 6 per cent to just over 2 per cent, the mean being 3.63 per cent. On the plats of high productivity, the range was from about 4.9 per cent to about 0.25 per cent, with the mean at 1.35 per cent.

The wide gaps between 1.4 per cent infection, 3.6 per cent infection, and 29 per cent infection are so striking as to leave no doubt of real differences.

⁷ Richards, B. L. Beet disease situation (in Utah) during 1921. Utah Farmer 18: May 20, May 27, 1922.

TABLE 2.—*Number of beets, number of diseased beets, and the percentage of beets infected with rootrot disease on plats of low, intermediate, and high soil productivity. Counts taken Sept. 15, 1926*

Low productivity plats					Intermediate productivity plats				High productivity plats			
Plat no.	Total no. beets	No. beets diseased	Per cent rootrot		Plat no.	Total no. beets	No. beets diseased	Per cent rootrot	Plat no.	Total no. beets	No. beets diseased	Per cent rootrot
6G	406	291	71.67		18G	657	23	3.50	25G	838	41	4.89
10G	733	91	12.41		19G	711	27	3.80	26G	748	12	1.61
17G	335	208	62.09		49G	728	20	2.75	28G	769	2	0.26
20G	460	132	28.70		83G	796	49	6.16	29G	833	2	0.24
35G	547	122	22.30		85G	810	26	3.21	30G	676	26	3.85
38G	435	87	16.51		93G	821	30	3.65	44G	861	3	0.35
39G	510	96	18.82		95G	814	18	2.21		668 ^a	23	3.44 ^a
40G	734	130	17.71						48G	840	10	1.19
46G	605	51	8.43						50G	875	13	1.49
47G	479	169	35.73						43F	790	7	0.89
54G	630	347	55.08						45F	835	7	0.84
55G	665	146	25.84						53F	885	11	1.24
									55F	710	5	0.70
									63F	864	9	1.04
									65F	790	6	0.76
									73F	857	5	0.58
									75F	927	4	0.43
Ave.	545	155.83	28.6			762	27.57	3.63		809	10.94	1.35

^a Infected with nematodes.

Even these wide differences, however, are only part of the story. The other part lies in the actual number of beets harvested. The plats were the same size and should have produced approximately equal numbers of roots. The mean expectation of beets on the well-manured plats is about 800 to the 1/25-acre. The mean of 809 beets on the plats of high productivity closely approaches this number. The mean of 762 beets on the plats of intermediate productivity shows a loss of 47 beets, or 6 per cent, as compared with the number on the highly productive plats. On the plats of low productivity there was a mean of only 545 beets, or a decrease of 264 beets, which is a loss of 33 per cent. These losses represent the number of beets which died early enough in the season not to be counted on September 15 when the official disease count was made. In other words, 33 per cent of the beets died and rotted early enough to have disappeared before September 15. In addition, 29 per cent of the remaining 67 per cent were observed to be diseased on that date. Computation shows this loss due to rootrot to be a total of about 52 per cent of the number of beets that should have been harvested. On the plats of intermediate productivity the total loss was about 9.5 per cent. The extent of loss due to rootrot was therefore about 1.5, 9.5, and 52 per cent on the plats of low, intermediate, and high productivity, respectively.

The epidemic of 1923 began early in the season, and the infected areas were more widespread. At harvest practically all roots were diseased on land low in productivity or poor in soil moisture, that is, either where soils were too dry or too wet. These conditions were studied on the Central Experimental Farm (North Logan) for soil productivity in the manner already described for 1926 as well as for soil moisture.

The total number of beets harvested about October 20, the number of diseased beets counted on October 8, and the percentage of diseased beets are shown in table 3. On the plats of low productivity practically all the plants were diseased, and many were dead and rotting on October 8. There were therefore more beets in many of the plats than were in a condition to be harvested two weeks later when the actual harvest was made. This is shown by more than 100 per cent being classified as diseased on some plats.

In the plats of low soil productivity there were only three of eleven plats where the percentage of diseased beets was not 100 or more. On the basis of total beets at harvest, the average was 102 per cent, which means that many beets rotted completely. In the plats of intermediate productivity the percentage diseased was 69, whereas it was only 5.3 in the plats of high productivity. In addition, there was a decrease in total number of beets of 16 and of 32 per cent for the plats of intermediate and low productivity, respectively. This means an infection of almost 100 per cent, of about 75 per

TABLE 3.—*Total number of beets, number of diseased beets, and percentage of beets injured by rootrot disease on plats of low, intermediate, and high soil productivity. Counts taken on October 8, 1923. Crop harvested October 20, 1923*

Low productivity plats				Intermediate productivity plats				High productivity plats			
Plat no.	No. plants diseased	Total no. beets at harvest	Per cent root-rot ^a	Plat no.	No. dead and diseased beets	Total no. beets at harvest	Per cent root-rot ^a	Plat no.	No. dead and diseased beets	Total no. beets at harvest	Per cent root-rot ^a
5G	904	844	107	14G	822	787	104	22G	27	958	3
9G	846	740	114	15G	842	836	101	23G	49	504	10
13G	765	564	136	37G	759	808	94	28G	6	875	1
16G	794	398	199	86G	74	944	8	29G	16	917	2
34G	847	846	100	88G	140	578	24	30G	40	838	5
35G	850	808	105	96G	745	44G	18	1092	2
36G	655	649	101	98G	666	45G	36	944	4
39G	192	688	28					48G	155	809	19
46G	140	581	24					50G	85	884	10
51G	518	328	158					46F	954
52G	392	335	117					48F	860
								56F	967
								58F	909
								66F	1022
								68F	985
								76F	923
								78F	974
Ave.	627	616	102		527	766	68.8		48	907	5.3

^a On basis of total number of beets at harvest.

cent, and of about 5 per cent respectively, on the plats of low, of medium, and of high soil productivity.

The differences in susceptibility between the beets on the three grades of plats are really greater than the figures indicate. Plats 14G and 15G really belong in the low-productivity group for the year 1923, as their treatments began only in 1922, previous to which the land was badly depleted by 11 years of continuous small-grain growing without manure. Similarly, plat 48G should be ranked in the intermediate group instead of the high group on account of its having been manured only twice—in 1922 and in 1923—after 11 years of depletion by small grains.

The current season (1927) has been rather favorable to sugar-beets and not an epidemic year for rootrot. However, there has occurred a light attack of rootrot. This is fortunate, as it has been possible to discern slight attacks, which in epidemic years, or in years generally unfavorable to beets, would probably not have been observed. At the time of writing, harvest was some time off and the total number of beets harvested was not available. The percentage attacked by rootrot is calculated on the total number of beets in the plats on October 4, when the count was made. As extremely few beets were dying, this number is accurate within a minute fraction of 1 per cent. The data for 1927 are given in table 4.

The degree of injury on individual beets on the plats of low productivity was much greater than on the other two groups. The differences between 42.8, 6.7, and 2.3 per cent for the plats of low, intermediate, and high productivity again conform to the effects in the epidemic years.

TABLE 4.—*Percentage of beets attacked by rootrot in plats of low, intermediate, and high soil productivity. Counts taken October 4, 1927. Percentage on basis of total number of beets just before harvest*

Low productivity plats		Intermediate productivity plats		High productivity plats	
Plat no.	Per cent diseased	Plat no.	Per cent diseased	Plat no.	Per cent diseased
7G	46.2	14G	9.6	26G	2.2
9G	13.5	15G	9.9	27G	4.9
13G	60.5	82G	5.4	28G	0.0
16G	26.7	84G	9.7	29G	0.6
34G	25.7	92G	2.9	30G	0.4
35G	31.2	94G	2.4	37G	5.9
36G	55.8			48G	3.1
39G	42.6			50G	1.5
53G	79.6				
54G	46.1				
Average	42.8		6.7		2.3

EFFECT OF SOIL MOISTURE

During the season of 1923 the rootrot developed early, that is, before and shortly after thinning time. Careful observations were made in the beet fields of the following districts: Logan, North Logan, Hyde Park, Smithfield, Lewiston, Benson, and College. The order given is that recorded on a trip circuit from the Experiment Station and return.

The disease showed in spots in some fields and as blanket infection in others. The field studies were limited to those fields in which there were spots of diseased beets and spots of healthy beets.

The data given in the fifth column of table 5 were obtained by averaging five counts in each field where the moisture conditions were unfavorable for the beets. The data in the sixth column represent averages of five counts in

TABLE 5.—*The effect of soil moisture on the development of rootrot of sugar-beets during the seedling stages. Observations made in July, 1923, in Cache Valley, Utah*

Locality	No. of acres	Occurrence of rootrot	Low soil moisture		Per cent rootrot where soil moisture was high
			Cause	Per cent rootrot	
1. Logan	3.5	Entire field	Spring-plowed alfalfa	87	0
2. Logan	8.0	Lower end only	Not well irrigated	54	18
3. Logan	4.5	Spots	Gravelly soil	61	9
4. Logan	4.0	Spots	Gravelly soil	49	13
5. Logan	1.5	One side	Coarse manure	73	6
6. North Logan...	12.6	Large spot	Gravel subsoil	59	8
7. North Logan...	11.0	Middle	Steep—water runs rapidly	70	3
8. North Logan...	7.0	Top half	Heavy grain stubble	46	7
9. North Logan...	2.5	Entire field	Delayed irrigation	55	0
10. North Logan...	15.0	Bottom only	Not well irrigated	65	14
11. North Logan...	5.0	Bottom only	Flooded at bottom	34	11
12. Hyde Park	20.0	Entire field	Poor packing	39	0
13. Hyde Park	4.0	One side	Cloddy soil	67	5
14. Hyde Park	13.5	Spots	No cause visible	78	21
15. Smithfield	3.0	Spots	Gravelly soil	39	12
16. Smithfield	3.0	Spots	Gravelly soil	61	7
17. Smithfield	7.0	Upper half	Steep slope	66	14
18. Smithfield	18.0	One side	Alfalfa sod	59	8
19. Lewiston	60.0	Large spots	Some high, some low and wet	85	20
20. Lewiston	20.0	Lower end	Wet land	90	10
21. Lewiston	22.0	Entire area	Recent plowing	63	9
22. Lewiston	9.0	Spots	Long grain stubble	44	14
23. Benson	10.0	9/10 of field	Wet land	99	15
24. Benson	11.5	Bottom half	Very compact—some alkali	75	15
25. College	30.0	3/4 of field	Wet land	50	4
26. College	14.5	1/2 of field	Alfalfa sod	35	2
27. College	5.5	Spots	Excessively compact	45	8
			Ave.	61.0	9.4

parts of the same fields where the moisture conditions were favorable for beets. The figures for each particular field therefore are directly comparable.

The data reported in table 5 indicate that six times as many beets were diseased where the moisture conditions were unfavorable for beets as where satisfactory moisture conditions prevailed.

More general observations were made in several other counties of the state. Actual counts were not taken, but careful general observations were made in several fields of each county and the percentage of rootrot estimated. These data correspond roughly with those obtained in Cache County by carefully replicated counts. Table 6 summarizes the estimated

TABLE 6.—*The effect of soil moisture on the development of rootrot of sugar-beets in several counties of Utah and one county of Idaho. (No field counts taken—farmers not consulted)*

County	No. fields	Per cent soil with unfavorable moisture (based on total area)	Per cent rootrot when soil moisture was unfavorable	Per cent rootrot when soil moisture was favorable
Boxelder	9	25	75	10
Weber	4	50	50	10
Davis	7	35	50	15
Salt Lake	4	40	60	20
Utah	18	20	50	15
Sanpete	3	5	20	2
Sevier	7	2	15	Trace
Franklin (Idaho) ..	7	30	35	5
Ave.			44.4	9.6

percentages of rootrot. There seems to have been almost five times as much disease in the spots of unfavorable moisture as in favorable spots.

There seems, therefore, to be good reason for concluding that unfavorable soil moisture conditions, particularly early in the season, predispose sugar-beets to attacks by rootrot.

Another set of data from the experimental plats also bears on this question of predisposition of sugar-beets to rootrot. Table 7 shows the effect of

TABLE 7.—*The effect of manure on development of rootrot in sugar-beets following other crops and following beets. Logan, Utah, 1923*

Crop rotation	Per cent rootrot	
	Manure	No manure
First year beets after other crop.....	94	8.0
Second year beets after other crop.....	51	3.0
Continuous beet crops.....	46	2.5

manure which is modified in a smaller way by the crop-rotation treatments.

There are great differences between manured and unmanured beets (Figs. 2 and 3). There is regularly about 12 to 15 times as much disease in beets on unmanured land. There was only about one-half as much disease when the beets followed beets as when beets followed other crops—in this case alfalfa, peas, and small grain. It is thought that the partially decomposed stubble residues from crops other than beets prevented optimum moisture or nitrate conditions from being established in the soil.

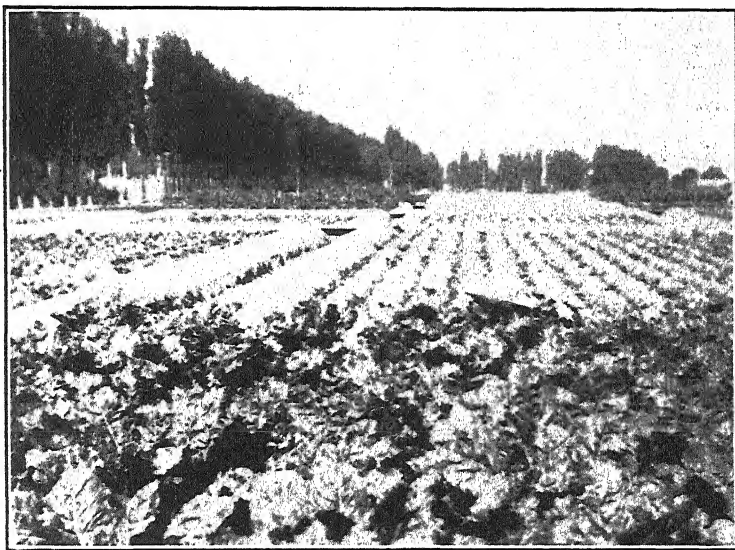


FIG. 2. Foreground: well manured sugar-beets in epidemic year. Back: poorly fertilized beets. All other conditions except soil treatment were equal.

SUMMARY AND CONCLUSIONS

1. Plats on the Central Experimental Farm of the Utah Experiment Station were grouped into classes of high, intermediate, and low productivity according to their yield of sugar-beets in non-epidemic years.

2. There were no differences in the degree of injury by curly-top on plats varying in soil productivity. Not nearly so many data were obtained regarding curly-top as regarding late rootrot.

3. There were, however, vast differences in the degree of injury by rootrot. These differences were permanent and outstanding. All plats low in soil productivity were heavily injured in both years when rootrot was bad. Plats of intermediate soil productivity were injured to an intermediate

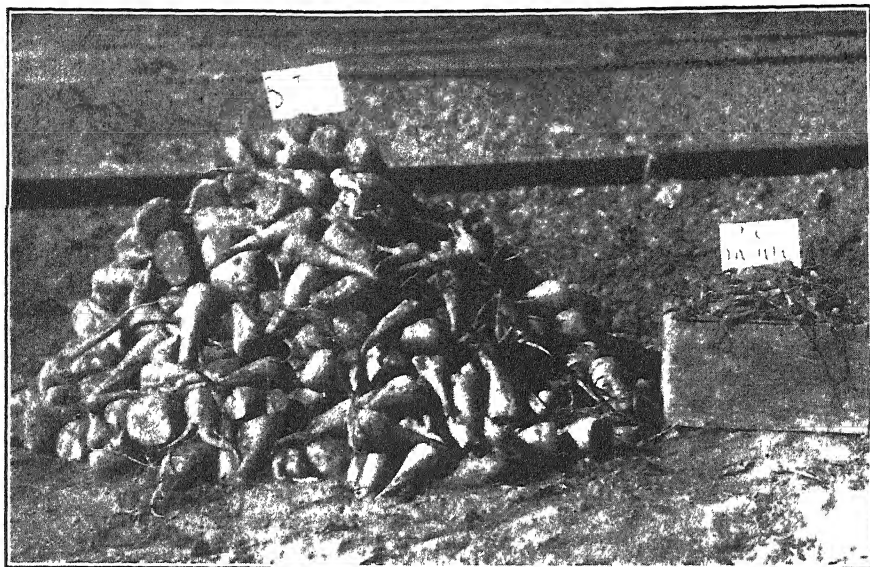


FIG. 3. Yield of sugar beets from 1/25-acre plats in 1921 at Central Experimental Farm, Logan, Utah. Left. actual beets from one entire plat heavily manured. Right: actual beets (on box) from one entire plat not manured at all. All other treatments were the same.

degree, and plats of high productivity were practically uninjured even when immediately adjacent, on every side, to plats fully infected.

4. Low productivity predisposes sugar-beets to the attack of rootrot so definitely and so strongly as to make good soil treatment, especially the use of farm manure and of reasonable crop rotation, the only control method the farmer needs to consider.

5. Unfavorable (either too dry or too wet) soil moisture conditions, especially early in the season, predisposed the beets to rootrot definitely, strongly, and almost without exception.

6. The maintenance of proper soil moisture conditions largely, if not entirely, overcomes this predisposition. In addition, soil moisture is more readily maintained in soils properly manured and rotated.

UTAH AGRICULTURAL EXPERIMENT STATION,
LOGAN, UTAH.

THE PRODUCTION OF AGGLUTININS BY PHYTO-PATHOGENIC BACTERIA¹

M. C. GOLDSWORTHY²

Researches in immunological and serological fields have been principally confined to animal life. The impetus to these researches has been the necessity of preserving human life and finding out the exact relations of antigens and antibodies to their hosts. The use of these serological methods in relation to plant diseases has been almost entirely neglected. Until recently workers have confined themselves to animal parasites in their relation to immune body formation.

Zipfel (7), as late as 1912, was probably the first to produce anti-sera by using phytopathogenic bacterial antigens. He was concerned with the relations of the various legume nodule bacteria, isolated from various hosts, to each other. Klimmer and Kreuger (4), working also with legume bacilli, found that from different species affected with the type of organism commonly known as *Bacillus radiculicola*, closely related organisms were obtained, which proved to be specific for the host from which they were isolated when treated with antisera of the blood of rabbits. That is, each host had a specific bacterium which closely resembled the type, *B. radiculicola*.

Jensen (3) produced antisera against forms of *Bacterium tumefaciens* S. and T., the widespread crown gall-causing organism. Paine and Lacey (5), by the use of antisera, showed the relationship of various organisms isolated from a diseased bean leaf. St. John-Brookes, Nain, and Rhodes (6) recently published a very excellent paper on the relations of many plant parasites to each other. Thirty-nine parasites were used. Antisera were produced against each, and cross agglutination was observed. These parasites came from various sources and fell into three groups, the yellow, white, and fluorescent forms. The investigators noticed considerable cross-agglutination among the members of each group, but not between the groups. Work of this nature will go a long way in clarifying the field of bacteriology as related to plant diseases. Very little is known as to the relations of the various similar plant disease organisms. There are many plant disease organisms that appear to be one and the same, but have been described as different species because they cause a disease on a different host.

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The above workers were all concerned with the relation of the specificity of isolated organisms. That organisms react so specifically with their antisera makes identification of these species absolute when the antisera are used in proper dilutions.

The writer's researches concern the bacterial organisms causing the gummosis of stone fruit trees. More than one organism appears to be active in producing this disease, although the literature on the subject describes only one species. Griffin (2) has described the organism *Pseudomonas cerasus* as causing this disease. The writer has discovered that at least two organisms, so closely related to each other that often it is impossible to tell them apart, are concerned in the disease formation. The writer designates these as *Ps. cerasus* var. 28 and 29. The only apparent difference is the production of a fluorescent pigment in liquid and solid media by one of them. This pigment is not always produced, and it is often difficult to separate the two organisms. Both cause the typical bacterial gummosis lesions when inoculated into healthy trees. As neither of these parasites could be easily distinguished, it became necessary to look for something more specific. If an agglutinating anti-serum could be produced against these parasites, then this method offered the means of differentiating and at the same time absolutely determining whether these were two species or whether they were the same organism. At the same time these antisera could be used in the study of the epidemiological relations. The persistence of the organisms in the soil—artificially and naturally, their persistence in old cankers, occurrence during the whole of the year in diseased trees, the part that certain orchard insects might have in the dissemination of these organisms and many other relations could be studied more readily with such sera, as all of the above studies would necessitate the collection of many bacterial cultures. The desired data could then be derived from the identification by use of the potent antisera.

The writer was also concerned with *Bacterium maculicolum* which causes the spot disease of cauliflower heads. This organism and the two varieties of *Ps. cerasus* causing the bacterial gummosis of stone fruit trees were used in the production of agglutinating antisera.

EXPERIMENTAL DATA

The production of an agglutinating antiserum is in itself normally not a difficult procedure. The methods of producing agglutinating antisera are numerous. The method used in these experiments follows that described by Gay and Fitzgerald (1).

Experiment I

Methods. The organisms were grown on Bacto-peptone-beef bouillon-agar slants, having a reaction of pH 6.8. The cultures were incubated at

room temperature. At the end of 48 hours, the resultant growth was suspended in 10 cc. of physiological salt solution (0.85 per cent). The suspension was then treated with enough 5.0 per cent phenol solution to bring the mixture to 0.25 per cent phenol. The phenolized cultures were allowed to stand for several hours before using, to allow the organisms to react with the phenol.

The injections were given intravenously in the amounts indicated in the animal charts. Preliminary bleeding to determine the course of the reaction was made from the marginal vein of the ear.

Rabbits were used for the experiment. Fairly large rabbits, weighing approximately 2,500 grams, gave the best results. One rabbit was used for each antigen. This experiment was a preliminary one, and the use of many rabbits seemed out of the question.

Results. The results were disappointing. In no case did the rabbits give potent antisera against the antigen. The experiment was carried on for almost a month, but during this time the titre did not reach a high point.

The cauliflower organism, *Bacterium maculicolum* McCulloch, apparently gives off a toxic substance. The first rabbit used against this antigen succumbed after five days. The organism used was dead, for on plates poured from the suspension there was no growth for 10 days. As a result of the death of this rabbit only the two gummosis organisms could be tested in the experiment. Charts 1, 2, and 3 show the course of the reactions.

Animal chart 1.

Rabbit 846.

Antigen, *B. maculicolum* (cauliflower organism) phenolized.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Jan. 26	<i>B. maculicolum</i>	0.5 cc. (i. v.)	Excellent	2800
Jan. 27	do	1.0 cc. (i. v.)	Good	2750
Jan. 28	do	1.0 cc. (i. v.)	Poor	2600
Feb. 1	Dead

Post mortem was made too long after death. The animal, before it died, had an emaciated appearance, clots of blood could be seen in the veins of the ear, and at the site of inoculation a large black necrotic area had formed. Stools that formed were hard.

Animal chart 2.

Rabbit 847.

Antigen, *Pseudomonas cerasus* 28; phenolized.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Jan. 26	28	0.5 cc. (i. v.)	Excellent	2200
Jan. 27	28	1.0 cc. (i. v.)	do	2100
Jan. 28	28	do	do	2100
Feb. 2	28	do	do	2200
Feb. 3	28	do	do	2250
Feb. 4	28	do	do	2200
Feb. 10	Bled from the heart	Titre, 1-160	do	2380
Feb. 11	28	1.0 cc. (i. v.)	do	2350
Feb. 16	28	1.5 cc. (i. v.)	do	2400
Feb. 17	28	do	do	2380
Feb. 18	28	do	do	2380
Feb. 24	Bled from ear	Titre, 1-160	do	2500
Feb. 25	Bled from heart	Titre, 1-320	do	2500

Normal serum obtained on the first day did not give a titre with the antigen.

Animal chart 3.

Rabbit 848.

Antigen, *Pseudomonas cerasus* 29; phenolized.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Jan. 26	29	0.5 cc. (i. v.)	Excellent	2100
Jan. 27	29	1.0 cc. (i. v.)	do	2000
Jan. 28	29	do	do	2120
Feb. 2	29	do	do	2130
Feb. 3	29	do	do	2120
Feb. 4	29	do	do	2120
Feb. 10	Bled from heart	Titre, 1-640	do	2180
Feb. 11	29	1.0 cc. (i. v.)	do	2200
Feb. 16	29	1.5 cc. (i. v.)	do	2230
Feb. 17	29	do	do	2260
Feb. 18	29	do	do	2260
Feb. 24	Bled from ear	Titre, 1-640	do	2240
Feb. 25	Bled from heart	Titre, 1-640		

Normal serum obtained on the first day did not give a titre with the antigen.

Phenolized suspensions of the two gummosis types failed to give appreciable titres. The cauliflower organism proved toxic to the rabbit and care was taken in the next experiment in the injections of this antigen. The experiment was repeated without phenolizing the antigens. Living antigens were used.

Experiment II

Methods. The titres of the antisera obtained during the first experiment were not high enough to give any specificity to the sera. It was thought that the use of killed cultures reduced the possibility of obtaining high titres. In the second experiment the procedure was changed. Instead of using killed cultures throughout, living cultures were used during the second week. Three animals were used as before. Care was taken in giving the injections of the cauliflower organism. Young rabbits were used this time.

Results. The results in general were far more satisfactory than those of the first experiment, but the titres were not what could be expected. Only one of the antisera gave a titre over 2000. The cauliflower organism again caused necrosis of the rabbit's ear, but the rabbit responded and overcame the initial reaction. Charts 4, 5, and 6 show the paths of the reactions with the different rabbits.

Animal chart 4.

Rabbit 902.

Antigen, *Bacterium maculicolum*; 3 injections of phenolized; 3 injections of living.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Mar. 16	<i>B. maculicolum</i>	1.0 cc. (i. v.)	Excellent	2005
do	Bled from ear	No titre		
Mar. 17	<i>B. maculicolum</i>	1.0 cc. (i. v.) dead	Excellent	2000
Mar. 18	do	do	Necrosis of ear	1950
Mar. 19	No change	1960
Mar. 23	<i>B. maculicolum</i>	1.0 cc. (i. v.) living	Excellent	2060
Mar. 24	do	1.25 cc. do	do	2050
Mar. 25	do	1.50 cc. do	do	2140
Mar. 26	do	2100
Mar. 30	Bled from the ear	Titre, 1-2560	do	2190
Mar. 31	Bled from carotid	Titre, 1-2560		

Final titre, 1-2560.

The results clearly show that the injections of living organisms stepped up the titre appreciably. The titres are still too low for specific use. The experiment was then repeated, with living cultures only.

Experiment III

Methods. The titres obtained during the course of the second experiment indicated that the use of living cultures throughout would give the

Animal chart 5.

Rabbit 903.

Antigen, *P. cerasus* 28; 3 injections of phenolized, 3 injections of dead cultures.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Mar. 16	28a	1.0 cc. (i. v.) dead	Excellent	1780
Mar. 17	28	do	do	1840
Mar. 18	28	do	do	1860
Mar. 19	1910
Mar. 23	28	1.0 cc. (i. v.) living	do	1950
Mar. 24	28	1.25 cc. do	do	2000
Mar. 25	28	1.50 cc. do	do	2020
Mar. 26	do	2040
Mar. 30	Bled from the ear	Titre, 1-1280	do	2100
Mar. 31	Bled from the carotid	Titre, 1-1280	do	2080

Final titre, 1-1280.

^aBled from the ear for normal blood titre. No titre.

Animal chart 6.

Rabbit 904.

Antigen, *P. cerasus* 29.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Mar. 16	29a	1.0 cc. (i. v.) dead	Excellent	1810
Mar. 17	29	do	do	1800
Mar. 18	29	do	do	1800
Mar. 19	do	1900
Mar. 23	29	1.0 cc. living	do	1960
Mar. 24	29	1.25 cc. do	do	2000
Mar. 25	29	1.50 cc. do	do	1950
Mar. 26	do	2060
Mar. 30	Bled from ear	Titre, 1-1280	do	2160
Mar. 31	Bled from carotid	Titre, 1-1280	do

Final titre, 1-1280.

^aNormal blood did not give a titre with the antigen.

expected results and that high specific serum titres could be obtained. In accordance with these observations the third experiment was conducted with living suspensions. The work was discontinued with the cauliflower antigen. Two rabbits were used for each of the gummosis organisms.

Results. Very good titres were secured during the course of the third experiment (Charts 7-10). Three of the rabbits responded with titres

above 5000, while the other rabbit gave a titre of only 1-2560. All were better than in the previous experiments. Animal 906 was carried through one more week than rabbit 905. At the end of the experiment the titre of rabbit 906 was not greater than that of 905. Small rabbits and large rabbits appear to react the same to this organism. At the end of the second week of injections the titres of the two rabbits were the same. Living cultures were used throughout and the results indicate that, in these two cases at least, living antigens have to be used in order that high-titre, potent sera can be obtained.

Animal chart 7.

Rabbit 905.

Antigen, *Ps. cerasus*, 2S; living cultures.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Apr. 6	2S	1.0 cc. living (i. v.)	Excellent	2910
do	Bled from ear	Normal serum, no titre	do	2910
Apr. 7	2S	1.0 cc. living (i. v.)	do	2870
Apr. 8	2S	do	do	2940
Apr. 13	2S	1.5 cc. living (i. v.)	do	3020
Apr. 14	2S	do	do	2960
Apr. 15	2S	do	do	3080
Apr. 20	Bled from ear	Titre, 1-5120	do	3300
Apr. 23	Bled from carotid	Titre, 1-5000	do	3360

Final titre, 1-5000.

Agglutination Experiments

Having produced these excellent titres, the two types were then subjected to cross agglutination experiments. As the organisms were so closely related, it was thought that they would cross agglutinate to some degree. Strange to say the organisms proved very specific: no cross agglutination was noted. *Pseudomonas cerasus* 2S and 29 are apparently distinct types causing the same disease. They did not cross agglutinate with the cauliflower organism, *B. maculicolum*. *Bacillus typhosus*, *Bacillus coli* and *Staphylococcus aureus* were subjected to the tests and they also failed to show any cross agglutination with the antisera of *Ps. cerasus*.

Methods. The agglutination titrations were carried out under the standard method as outlined in Kolmer's text. Agar streak cultures, 24 hours old, were suspended in physiological salt solution so that the suspension had a turbidity equal to that of a standard tube which should contain 2,000,000,000 units per cc. The writer used a standard furnished by Dr.

Animal chart 8.

Rabbit 906.

Antigen, *Ps. cerasus*, 28; living cultures.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Apr. 6	28	1.0 cc. (i. v.) living	Excellent	1820
do	Bled from ear	Normal blood, no titre	do	1820
Apr. 7	28	1.0 cc. (i. v.) living	do	1740
Apr. 8	28	1.5 cc. (i. v.) living	do	1780
Apr. 13	28	do	do	1940
Apr. 14	28	do	do	1860
Apr. 15	28	do	do	1880
Apr. 20	Bled from ear	Titre, 1-5120	do	2050
Apr. 23	28	2.0 cc. (i. v.) living	do	2150
Apr. 24		.	do	2080
Apr. 26		.	do	2200
Apr. 28		.	do	2020
May 1	Bled from ear	Titre, 1-5120	do	2150
May 4	Bled from carotid	Titre, 1-5120	do	2320

Final titre, 1-5120.

Animal chart 9.

Rabbit 907.

Antigen, *Ps. cerasus*, 29; living cultures.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Apr. 6	Bled from ear	Titre, 0	Excellent	1910
do	29	1.0 cc. (i. v.) living	do	1910
Apr. 7	29	do	do	1910
Apr. 8	29	1.5 cc. (i. v.) living	do	2020
Apr. 13	29	do	do	2060
Apr. 14	29	do	do	2000
Apr. 15	29	do	do	2060
Apr. 20	Bled from ear	Titre, 1-5120	do	2060
Apr. 23	Bled from carotid	Titre, 1-5120	do	2080

Final titre, 1-5120.

Alvarez. The standard was duplicated and used for the remainder of the experiment. One half of one cc. standard suspension was added to each 0.5 cc. of diluted serum, in saline. The mixture was allowed to stand in a water bath for two hours at 37° C. Readings were made each hour. At

Animal chart 10.

Rabbit 908.

Antigen, *Ps. cerasus*, 29; living cultures.

Date, 1925	Antigen	Amount of antigen	Appearance of rabbit	Weight in gms.
Apr. 6	Bled from ear	Titre, 0	Excellent	2150
do	29	1.0 cc. (i. v.) living	do	2150
Apr. 7	29	do	do	2050
Apr. 8	29	1.5 cc. (i. v.) living	do	2160
Apr. 13	29	do	do	2160
Apr. 14	29	do	do	2200
Apr. 15	29	do	do	2220
Apr. 20	Bled from ear	Titre, 1-5120	do	2280
Apr. 23	29	2.0 cc. (i. v.) living	do	2320
Apr. 24	do	2250
Apr. 26	do	2400
Apr. 28	do	2210
May 1	Bled from ear	Titre, 1-2560	do	2360
May 4	Bled from carotid	Titre, 1-2560	do	2460

Final titre, 1-2560.

Titre dropped off after last injection was given. The reason for this is not apparent since this phenomenon did not occur in the rabbit so treated with antigen 28. The rabbit lost weight rapidly after the last injection and possibly some of the agglutinins were used up in the defense.

the end of the two hours the tubes were placed in the ice-box and then read the following morning. Saline plus antigen and serum were used throughout the experiment as checks.

Agglutination tests were made with the normal sera and immunized sera. Cross agglutination was tested with the immunized sera of the three antigens.

Results. In tables 1 and 2 the relation of the antigens to the homologous sera and to the normal sera are noted. Table 3 gives the cross agglutinating values of the different sera.

DISCUSSION AND CONCLUSIONS

The production of satisfactory agglutinating antisera against phytopathogenic bacteria appears to be as simple as against animal parasitic bacteria. Apparently the phytopathogenic forms differ greatly in their power to produce antibodies in the sera of rabbits. This power seems to be correlated with the toxic products that are elaborated.

TABLE 1.—*The results of agglutination tests with normal sera of rabbits 905, 906, 907, and 908 and the antigens of Pseudomonas cerasus 28 and 29. Tests made April 8, 1925.*

Serum and antigen	Dilutions								Time and temperature
	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	
Serum 905	-	-	-	-	-	-	-	-	1 hr., 37° C.
+	-	-	-	-	-	-	-	-	2 hrs., 37° C.
Antigen 28	-	-	-	-	-	-	-	-	18 hrs., 8° C.
Serum 906	-	-	-	-	-	-	-	-	1 hr., 37° C.
+	-	-	-	-	-	-	-	-	2 hrs., 37° C.
Antigen 28	-	-	-	-	-	-	-	-	18 hrs., 8° C.
Serum 907	-	-	-	-	-	-	-	-	1 hr., 37° C.
+	-	-	-	-	-	-	-	-	2 hrs., 37° C.
Antigen 29	-	-	-	-	-	-	-	-	18 hrs., 8° C.
Serum 908	-	-	-	-	-	-	-	-	1 hr., 37° C.
+	-	-	-	-	-	-	-	-	2 hrs., 37° C.
Antigen 29	-	-	-	-	-	-	-	-	18 hrs., 8° C.

TABLE 2.—*The results of agglutination tests with the antisera of rabbits 905, 906, 907, and 908 and the antigens of Pseudomonas cerasus 28 and 29*

Antiserum and antigen	Dilutions								Time and temperature
	1/320	1/640	1/1280	1/2560	1/5120	1/10000	Ck.	Ck.	
Antiserum 905	+	+	+	+	-	-	-	-	1 hr., 37° C.
+	+	+	+	+	+	-	-	-	2 hrs., 37° C.
Antigen 28	+	+	+	+	+	-	-	-	18 hrs., 8° C.
Antiserum 906	+	+	+	+	-	-	-	-	1 hr., 37° C.
+	+	+	+	+	+	-	-	-	2 hrs., 37° C.
Antigen 28	+	+	+	+	+	-	-	-	18 hrs., 8° C.
Antiserum 907	+	+	+	+	-	-	-	-	1 hr., 37° C.
+	+	+	+	+	+	-	-	-	2 hrs., 37° C.
Antigen 29	+	+	+	+	+	-	-	-	18 hrs., 8° C.
Antiserum 908	+	+	+	+	-	-	-	-	1 hr., 37° C.
+	+	+	+	+	-	-	-	-	2 hrs., 37° C.
Antigen 29	+	+	+	+	-	-	-	-	18 hrs., 8° C.

From the results of the experiments, it appears that, in the case of some phytopathogenic bacteria, production of suitable antisera can only be accomplished by the injection of living organisms in fairly large doses. In the

TABLE 3.—*The results of direct and cross agglutination tests with antisera of rabbits 902, 905, and 907, the antigens of Pseudomonas cerasus 28 and 29, and the antigen of Bacterium maculicolum*

Antiserum and antigen	Dilutions								Time and temperature	
	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280		Ck.
Antiserum 905	+	+	+	+	+	+	+	+	—	1 hr., 37° C.
+	+	+	+	+	+	+	+	+	—	2 hrs., 37° C.
Antigen 28	+	+	+	+	+	+	+	+	—	18 hrs., 8° C.
Antiserum 907	—	—	—	—	—	—	—	—	—	1 hr., 37° C.
+	—	—	—	—	—	—	—	—	—	2 hrs., 37° C.
Antigen 28	—	—	—	—	—	—	—	—	—	18 hrs., 8° C.
Antiserum 907	+	+	+	+	+	+	+	+	—	1 hr., 37° C.
+	+	+	+	+	+	+	+	+	—	2 hrs., 37° C.
Antigen 29	+	+	+	+	+	+	+	+	—	18 hrs., 8° C.
Antiserum 905	—	—	—	—	—	—	—	—	—	1 hr., 37° C.
+	—	—	—	—	—	—	—	—	—	2 hrs., 37° C.
Antigen 29	—	—	—	—	—	—	—	—	—	18 hrs., 8° C.
Antiserum 902	+	+	+	+	+	+	+	+	—	1 hr., 37° C.
+	+	+	+	+	+	+	+	+	—	2 hrs., 37° C.
Antigen <i>B. maculicolum</i>	+	+	+	+	+	+	+	+	—	18 hrs., 8° C.
Antiserum 905	—	—	—	—	—	—	—	—	—	1 hr., 37° C.
+	—	—	—	—	—	—	—	—	—	2 hrs., 37° C.
Antigen <i>B. maculicolum</i>	—	—	—	—	—	—	—	—	—	18 hrs., 8° C.
Antiserum 907	—	—	—	—	—	—	—	—	—	1 hr., 37° C.
+	—	—	—	—	—	—	—	—	—	2 hrs., 37° C.
Antigen <i>B. maculicolum</i>	—	—	—	—	—	—	—	—	—	18 hrs., 8° C.

case of the species that produce toxic products, extremely potent antisera can be hoped for. Titres against the ordinary forms cannot be expected to go higher than 1–10,000.

The writer feels that more work of this nature should be undertaken by plant as well as animal bacteriologists. Serological applications will go a long way in placing plant bacteriology on a sound basis. Too many of our phytopathogenic species are described without any effort to compare with known forms. Many of our pathogenic species closely resemble each other not only in the disease manifestation but in their morphological and biochemical characteristics. Antisera can be used not only in identification work but in studying epidemiology. Little is known about the dissemination, persistence and many other phases of our pathogenic forms. Possibly the greatest drawback to the plant bacteriological science has been the lack

of some means of identification without resort to inoculation experiments. On the successful inoculation rests the fate of many isolated organisms. Successful inoculations are rather rare in comparison with unsuccessful. Too much depends upon the condition of the host and the parasite as to whether an inoculation will be successful or not. The use of a highly potent antiserum should eliminate a great deal of the inoculation work and inconsistency in results.

SUMMARY

1. The literature on the production of antisera against phytopathogenic bacteria is discussed.

2. Experiments upon the production of antisera against three phytopathogenic forms, *Pseudomonas cerasus* var. 28, *Ps. cerasus* var. 29 (two bacterial gummosis types), and *Bacterium maculicolum*, an organism causing the spot disease of cauliflower, were conducted.

3. Potent antisera were produced against the three forms.

4. Living antigens gave the best results when injected intravenously.

5. Cross agglutination was not encountered.

The writer is indebted to Dr. K. F. Meyer and to Dr. Ruth Alvarez for their counsel and assistance in the preparation of the antisera.

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NOTES ON THE CROWNGALL SITUATION IN ENGLAND, FRANCE AND HOLLAND¹

A. J. RIKER

A survey of the crowngall situation in England, France, and Holland was made in the fall of 1926 in order to determine whether or not any correlation existed between the situation in these countries and that in the United States. The survey was undertaken in connection with recent work in the United States which has shown that a considerable number of the enlargements formed on certain plants, particularly nursery apple trees, were caused by other agencies than *Bacterium tumefaciens* Smith and Town. Miss Brown (1) has shown that the aerial stem tumors on apple, which were sometimes called burr knots, were not crowngall. Riker and Keitt (6), Melhus (3), and Muncie (4) reached the conclusion that, while true crowngall was widespread, a very considerable percentage of the enlargements at the crown of nursery apple trees followed mechanical injuries or were incident to the grafting process. In Europe also, Wormald and Grubb (8) have concluded that the crowngall organism was not the only factor in the development of these enlargements. In view of this revision of the crowngall problem it seemed desirable to correlate the actual situation and the work which was being done on this subject both in America and Europe.

The methods employed in making this survey were similar to those described by Riker and Keitt (6) for the United States. Representative nurseries in England, France, and Holland in which apple trees and other susceptible stock were being grown were visited in the fall of 1926 and the plants examined as they were dug for shipment. When it was impractical to examine very large numbers of individual plants the percentages were secured from counts on several hundred plants taken at random. As the writer did not have facilities for making extensive cultural examinations of the material, the diagnosis was dependent upon the characteristics of crowngall and of wound overgrowth as described by Riker and Keitt (6). However, as they have shown, these characters are sufficiently distinctive so that a very high degree of accuracy may be secured.

In consideration of the results of this survey and in correlating them with the conditions in the North-central and Northeastern United States it is important to hold in mind several important factors which are different

¹ Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

certain sections of Europe from those in the United States. Notable among these are differences in environmental conditions, in the varieties of plants cultivated, and in the cultural practices employed.

The environmental conditions in England, Holland, and Northwestern France are in some respects quite unlike those in North-central and Northern United States. The latitude in this section of Europe provides for very much longer day in the summer season when the plants are making major portion of their growth and for very short days in the winter when the plants are dormant. At the same time the temperature is moderated so that burning hot days in the summer and very severe frost in the winter, which always occur in this section of the United States, are practically unknown. The moisture is supplied in a particularly favorable way. While the actual precipitation may, in this section of Europe, not be any greater, the average relative humidity of the air is considerably above that of this section of the United States, and the actual rainfall is distributed in frequent gentle showers which tend to keep the ground relatively moist. These and other factors combine to make the growing season considerably longer. At the same time the comparatively cool temperature appears to be influential in preventing the very rapid type of plant growth which is common during the spring and early summer in this section of America. Of course all these factors which influence plant growth are also important in the development of disease (see Jones *et al.* 2). The influence of certain environmental factors on the development of crown gall has been described by Riker (5).

The varieties of susceptible plants grown in this section of Europe are different in many cases from those grown in this section of the United States. It is quite apparent that there is a much greater requirement for fruit trees of small size in Europe. The comparatively high price of land, small portions held by one individual, and the comparatively cheap labor have developed a very intensive cultivation. It appears that this situation is largely responsible for the great popularity, in some regions, of Doucin and Paradise apple stock because the trees are more or less dwarfed and bear fruit at an earlier age. At the same time the climate in this section of Europe, with its comparatively mild winters and cool summers, favors the propagation of some varieties which would not live under certain American conditions. While exact information on the relative susceptibility to crown gall of different types of fruit trees is lacking, especially as it has been shown by several workers, as mentioned above, that many of the enlargements formerly called crown gall are not due to *Bact. tumefaciens*; nevertheless it is an important factor to be considered.

The method of propagation of fruit trees has been found to be very important in the incidence of wound overgrowth. In North-central United

States, of apple trees of the Wealthy variety which were propagated by piece root grafts, there was often 35 per cent with wound overgrowths (6), while the same variety which was propagated in adjacent rows by budding showed less than 3 per cent. Inasmuch as the piece-root graft for the propagation of apple trees, which is very common in the North-central United States, is quite unusual in Europe, it is not to be expected that European budded stock should show so much wound overgrowth.

Certain enlargements which are characteristic of particular varieties have not been mentioned in this survey. No record was taken of the presence of burr-knots which appear almost always on Paradise, Doucin, and certain other stocks. Although they were at one time considered to be crowngall, it has been shown by Brown (1) that they are not due to *Bact. tumefaciens* and by Swingle (7) that they are characteristic of certain varieties of stock.

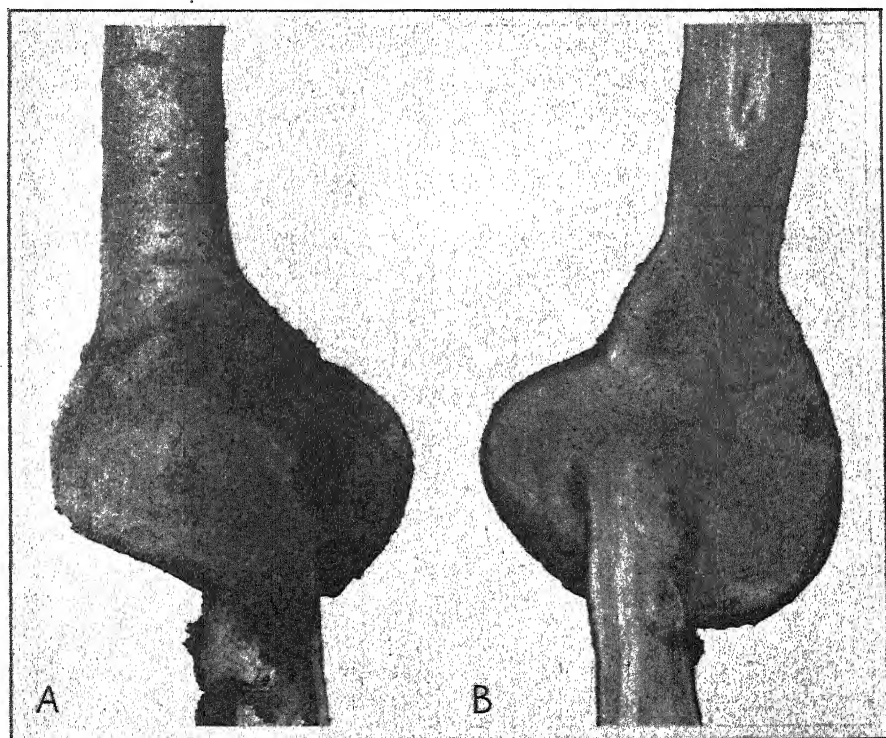


FIG. 1.—Enlargements formed in four years at the unions where vigorous apple scions were budded on dwarfing stocks. A. Surface. B. Section. $\times 2/5$. These specimens were provided by M. B. Crane and photographed by H. C. Osterstock, of the John Innes Horticultural Institution.

TABLE 1.—*Summary of the findings by a survey in England, France, and Holland, during the fall of 1926 for crown-gall and wound overgrowth on nursery stock*

Nurs- ery	Kind of stock	Wound over- growth ^a (per cent)	Crown- gall (per cent)	Nurs- ery	Kind of stock	Wound over- growth ^a (per cent)	Crown- gall (per cent)
<i>England</i>							
A	Apple	5	0	A con.	Raspberry	0	10 ^b
	do. seedling	1	0	B	Apple	6	trace
	Peach	0	0		Cherry	0	0
	Pear	trace	trace		Plum	0	0
	Plum	0	0		Raspberry	0	±
<i>France</i>							
A	Apple	10	0	D con.	Pear	0	0
	do. seedling	trace	0	E	Apple	0	0
	Pear	10	0		Cherry	0	0
	Rose	0	0		Pear	0	0
B	Apple	8	0		Rose	0	0
	do. seedling	trace	0	F	Apple	0	0
	Cherry	0	0		do. seedling	0	0
	Quince	1	0		Cherry	0	0
C	Apple	10	0		Pear	0	0
	Pear	trace	0	G	Apple	0	0
	Plum	0	0		Pear	0	0
D	Apple	trace	0		Rose	0	0
	do. seedling	0	0	H	Apple	0	0
	Cherry	0	0		Pear	0	0
	Peach	0	0		Plum	0	0
<i>Holland</i>							
A	Apple	8	0	E	Rose	1/10	0
	Cherry	12	0	F	Apple	1	0
	Rose	trace	0		Rose	trace	0
B	Rose	1/10	trace ^c	G	Apple		
C	Apple	70	0		seedling	0	0
	Rose	trace	0		do.	trace ^c	0
D	Apple	2	6	H	Apple	4	10
	Pear	60	10 ^d		Pear	5	15

^a This class includes excess callus, wound overgrowths, and non-infectious hairy-root. However, burr-knots, which are normal on Paradise, Doucin, and certain other stocks are not included.

^b One lot of raspberries had 40 per cent crown-gall while three other lots were entirely free from this disease.

^c These figures are based on examination of representative samples of 250,000 Monetti rose stocks intended for shipment to America. No crown-gall was found in the stock being prepared for shipment. In the pile of rejects only one plant was found which bore an enlargement resembling crown-gall.

^d The diagnosis is doubtful. A number of the plants bore enlargements which were typical of neither wound overgrowth nor crown-gall. Similar enlargements have been observed on pears grown on drained swamp land in Michigan.

* Callus and wound overgrowths were found only on transplanted seedlings.

Likewise no record was taken of such enlargements as those which very commonly appear at the union of apple trees budded on dwarfing stock (Fig. 1). The presence of these enlargements on such trees and their non-parasitic nature is common information in regions where this type of stock is grown. Their development is ordinarily explained on the basis of lack of congeniality between stock and cion. Ordinarily the budding in such cases is done sufficiently high so that these enlargements are several inches above the soil line. This is necessary because, where the enlargement touches the soil, it sends out a large number of roots (one type of hairy-root), and such a tree, becoming established on roots from the cion, is no longer dwarfed by the stock. It appears that the question of congeniality between stock and cion is important and should be considered in efforts to eliminate wound overgrowths from piece-root apple grafts.

A summary of the findings of this survey is given in table 1. In England and Holland, crowngall was an important factor in only three or four places. In each of these cases the size of the affected planting was so small that the owner was not concerned about any loss. In each of these instances also the owner never sold any of his plants in the United States. In Northwestern France no crowngall was observed. Both the nurserymen and the nursery inspectors are familiar with the disease and report that, while they find it from time to time, it is never of any serious consequence. Wound overgrowth was found in limited quantities in all the countries visited. When allowance is made for differences in climate, varieties cultivated, horticultural practices, and so forth, it appears that the crowngall and wound overgrowth situations in these European sections are very similar indeed to those in the North-central and Northeastern United States. In all these regions it appears that the malformations encountered in the underground parts of the nursery stock examined were predominantly callus, hairy-root, or wound overgrowths. Although it seems that infections by *Bact. tumefaciens* appear from time to time in all regions visited, the economic importance of true crowngall, except in isolated cases, is commonly very slight.

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EXPERIMENTS ON THE CONTROL OF BARLEY STRIPE¹

H. A. RODENHISER

INTRODUCTION

Attempts have been made recently to improve methods of treating barley seed for controlling barley stripe, *Helminthosporium gramineum* Rabh. The modified hot water method is effective but cumbersome, and the long-time soak in formaldehyde is not always effective. There also is some evidence that formaldehyde sometimes injures barley seed. Leukel, Dickson, and Johnson² have shown that some of the organic mercury compounds, including certain dusts, are quite effective against stripe. For several years the writer has been testing the effectiveness of these compounds, and the results are presented in this paper.

The control of barley stripe is assuming greater importance in Minnesota, as the amount of the disease seems to be increasing. This may be due to the fact that some of the varieties recently produced and distributed in Minnesota are quite susceptible to the disease. Minsturdi, Minn. 439, which is replacing to some extent Manchuria, Minn. 184, is quite susceptible. From 5 to 40 per cent of the plants of this variety usually are infected. There is some evidence, also, that the disease is increasing in certain other varieties grown in the State, especially Svansota, Minn. 440. This may be because of the gradual accumulation of inoculum due to the growing of susceptible varieties, or because of the increase of a more virulent strain of the pathogene. Johnson's work³ indicates that there may be several physiologic forms of *H. gramineum* in the State. Whatever the cause, it is perfectly evident that the disease is becoming more destructive, and it is imperative that control measures be simplified and improved.

The literature on the control of barley stripe has been reviewed thoroughly by Leukel, Dickson, and Johnson, and will therefore not be reviewed in this paper.

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² Leukel, R. W., James G. Dickson and A. G. Johnson. Experiments with dusts for controlling stripe disease of barley. *Phytopath.* 17: 175-179. 1927.

Leukel, R. W., James G. Dickson and A. G. Johnson. Seed treatment experiments for controlling stripe disease of barley. *Phytopath.* 16: 565-576. 1926.

³ Johnson, T. Studies on the pathogenicity and physiology of *Helminthosporium gramineum* Rabh. *Phytopath.* 15: 796-804. 1925.

MATERIALS AND METHODS

Both liquid and dust fungicides were used in the tests for the control of the stripe disease of barley. The chemicals used and the names of the manufacturers are as follows:

Bayer Dust	}	Bayer Company, Inc., New York City.
Uspulun		
Höchst		
Semesan	}	E. I. du Pont de Nemours and Company, Wilmington, Delaware.
Semesan Dust		
Du Pont No. 12		
K-I-A		
K-I-B		
Germisan	}	Saccharin-Fabrik, Aktiengesellschaft, vorm. Fahlberg, List and company, Magdeburg, Südost, Germany.
S. A. F. No. 225.....		
Wa Wa Dust		Chicago Process Company, Chicago, Illinois.
Coppercarb		Pittsburgh Plate Glass Company, Corona Chemical Division, Milwaukee, Wisconsin.
Kolo Dust		Niagara Sprayer Company, Middleport, N. Y.
Formaldehyde		Roessler and Hasslacher Chemical Company, Perth Amboy, New Jersey.

Minsturdi barley (Minn. 439) was soaked in Uspulun, 0.25 per cent solution; in Germisan, 0.25 per cent solution; Semesan, 0.3 per cent solution; and formaldehyde, 1-320. The periods of soak were one, two, and three hours at ordinary temperatures, and one half, one, and two hours at 45° C. Formaldehyde was also used as a dip, and the seed subsequently covered for five hours. Numerous plots were sown with untreated seed for comparison. The dust fungicides were used at the rate of 4 ounces to a bushel of seed. The dust and seed were shaken together in a 2-liter Erlenmeyer flask until the seed was thoroughly coated with a fine layer of dust. All of the liquid treatments were made so that the seed was thoroughly dry three days before planting. In 1925 and 1926, when yield data were taken, the seed was sown in triplicate eighteen foot rows in plots which were replicated three times. Counts on individual culms and yield data were taken from the center row of the triplicate plots. The approximate number of culms per row was between four hundred and four hundred and fifty. In 1924 and 1927 the seed was sown in individual rod rows in series of three and four, respectively.

TABLE 1.—*Effect of liquid fungicides on percentage of stripe (1924-1927) and on yield (1925-1926) of Minsturdi barley at University Farm, St. Paul, Minnesota*

Treatment		Acre yields			Percentage of culms infected				
		1925	1926	Average	1924	1925	1926	1927	Average
Uspulun, 0.25 per cent solu- tion	1 hour cold	66.0	62.5	64.3	tr. ^a	0.8	0.6	0.5	0.5
	2 hours cold	70.7	61.8	66.3	tr.	tr.	0.0	—	tr.
	3 hours cold	69.6	61.7	65.7	0.0	tr.	0.0	—	tr.
	$\frac{1}{2}$ hour at 45° C.	79.1	57.5	68.3	0.8	tr.	0.5	—	0.4
	1 hour at 45° C.	72.7	67.3	70.0	0.0	tr.	0.0	—	tr.
	2 hours at 45° C.	67.7	61.4	64.6	0.0	0.0	0.0	—	0.0
Germisan, 0.25 per cent solu- tion	1 hour cold	71.3	58.6	65.0	2.8	0.0	2.2	tr.	1.3
	2 hours cold	77.0	61.0	69.0	1.8	0.0	0.7	—	0.8
	3 hours cold	72.3	62.3	67.3	0.8	0.0	1.0	—	0.6
	$\frac{1}{2}$ hour at 45° C.	75.9	59.7	67.8	0.7	0.0	0.8	—	0.5
	1 hour at 45° C.	71.3	64.3	67.8	tr.	0.0	0.4	—	0.1
	2 hours at 45° C.	65.2	61.2	63.2	0.0	0.0	0.3	—	0.1
Semesan, 0.3 per cent solu- tion	1 hour cold	75.9	63.7	69.8	tr.	0.7	0.5	1.0	0.6
	2 hours cold	75.3	68.5	71.9	0.5	0.5	0.3	—	0.4
	3 hours cold	74.7	60.6	67.7	tr.	tr.	0.4	—	0.1
	$\frac{1}{2}$ hour at 45° C.	72.5	58.9	65.7	0.0	tr.	0.5	—	0.2
	1 hour at 45° C.	71.6	63.7	67.7	0.0	tr.	0.5	—	0.2
	2 hours at 45° C.	77.2	61.5	69.4	0.0	0.0	0.0	—	0.0
Formalde- hyde, 1-320	Dip, cover 5 hours	63.9	55.8	59.9	2.5	10.6	6.6	—	6.6
	1 hour cold	61.4	69.9	65.7	0.3	4.2	2.3	6.6	3.4
	2 hours cold	65.7	64.8	65.3	0.4	2.5	1.5	—	1.5
	3 hours cold	67.7	58.7	63.2	0.8	2.7	1.8	—	1.8
	$\frac{1}{2}$ hour at 45° C.	56.2	65.6	60.9	0.3	4.2	2.3	—	2.3
	1 hour at 45° C.	54.9	70.5	62.7	0.0	3.1	1.6	—	1.6
Control, tap water	2 hours at 45° C.	25.2	56.6	40.9	0.0	2.0	1.0	—	1.0
	Dry	64.0	56.2	60.1	13.2	11.2	10.1	6.7	10.3
	1 hour cold	66.2	67.7	62.0	—	10.5	11.4	6.6	9.5
	2 hours cold	65.1	57.8	61.5	—	9.8	10.9	—	10.4
	3 hours cold	67.6	58.0	62.8	—	9.1	9.2	—	9.2
	$\frac{1}{2}$ hour at 45° C.	70.4	63.6	67.0	—	4.4	5.7	—	5.1
	1 hour at 45° C.	71.5	58.2	64.9	—	3.5	2.1	—	2.8
	2 hours at 45° C.	75.8	64.7	70.3	—	1.8	3.6	—	2.7

^a tr. = trace of infection.

RESULTS

The results of treating barley with liquid fungicides to control barley stripe and the yield data are presented in table 1.

It was found that the effectiveness of the liquid fungicides used depended somewhat on the temperature and length of soak. Solutions of Uspulun and Semesan reduced barley stripe to 1 per cent or less for a period of years when the seed was treated for one hour at room temperature. In the corresponding control plot an average of 9.5 per cent of stripe developed. When the period of soak was increased to two and three hours,

Uspulun eliminated the stripe or reduced it to a trace, while Semesan reduced it to 0.5 per cent or less. An average of 10.4 and 9.2 per cent of stripe developed in the plots sown with seed soaked in tap water for two and three hours, respectively. The results obtained with Germisan were conflicting. In 1925 it eliminated the stripe under all the conditions tried, and in 1927 it reduced stripe to a trace. In 1924 and 1926, however, Germisan was not so effective as Uspulun or Semesan when the seed was soaked in the solution at ordinary temperatures. One-half hour treatment in Uspulun, Germisan, and Semesan, at 45° C., reduced the stripe to less than 1 per cent as compared with an average of 5.1 per cent of stripe in the control plot. Germisan and formaldehyde caused delayed heading of the grain in 1924 and 1925 when the period of soak at 45° C. was extended to one and two hours. Formaldehyde dip reduced the amount of stripe but not sufficiently for practical purposes. When used at room temperature for the various periods of soak it controlled the stripe in 1924. In other years formaldehyde reduced the stripe but was not so effective as the organic mercury compounds tested. Hot formaldehyde caused marked injury to the stand in 1924.

In no instance did the plants of individual plots emerge from the soil early enough to warrant the statement that the fungicide stimulated germination of the seed. Yield data were taken in 1925 and 1926. In comparing the yield data in the individual years, there were apparently no significant differences in the yields obtained from plots treated with the organic mercury compounds. Formaldehyde reduced the yields in 1925 but had no apparent effect on them in 1926.

The results of the dust treatments are given in table 2. Four of the ten dusts used controlled stripe satisfactorily. K-I-A and K-I-B eliminated the stripe in all four series, while Du Pont No. 12, S. A. F. No. 225, and Wa Wa Dust reduced it to an average of 0.4 per cent or less. These dusts, therefore, appear very promising, although their effect on yields was not determined. However, K-I-A and K-I-B were used by the writer for treating oats and hullless barley against smut and did not decrease yields. Bayer Dust, Semesan Dust, and Höchst reduced the stripe to less than 2 per cent, while the Coppercarb and Kolo Dust were ineffective in controlling the stripe.

DISCUSSION

It is evident that several of the organic mercury compounds, notably Uspulun, Semesan, and Germisan, when used in solution, are preferable to formaldehyde for controlling barley stripe. All of these fungicides controlled stripe quite effectively when used in cold, as well as in hot, solution. In addition, the yields were better than those obtained from untreated

TABLE 2.—*The effect of chemical dusts on the percentage of stripe in Minsturdi barley at University Farm, St. Paul, Minnesota, in 1927*

Fungicide ^a	Percentage of infected culms				
	Series				Average
	A	B	C	D	
Coppercarb	4.2	4.4	5.2	4.8	4.7
Kolo Dust	5.3	5.3	4.8	7.8	5.8
Bayer Dust	2.3	2.0	0.7	1.2	1.6
Semesan Dust	2.0	tr.	tr.	2.3	1.1
Untreated	7.0	5.2	7.4	7.2	6.7
Wa Wa Dust	tr. ^b	0.7	0.0	tr.	0.2
Höchst	2.9	0.7	1.2	2.2	1.8
S. A. F. No. 225	tr.	0.0	0.7	tr.	0.2
Du Pont No. 12	tr.	0.0	tr.	1.5	0.4
Untreated	6.9	7.2	7.5	7.5	7.3
K-I-A	0.0	0.0	0.0	0.0	0.0
K-I-B	0.0	0.0	0.0	0.0	0.0

^a Applied at the rate of 4 oz. a bushel of seed.

^b tr. = trace of infection.

grain, although there was no evidence that the fungicides had any special stimulatory effect. Formaldehyde was not so effective as these organic mercury compounds and also had a tendency in some cases to reduce yields. It seems safe to conclude, therefore, that the organic mercury compounds are preferable to formaldehyde. And they certainly are preferable to the modified hot-water treatment because of the saving of time and labor.

Certain of the dust fungicides were surprisingly effective, but their effect on yields is not yet known. However, the writer has treated oats and hulless barley with some of these dusts, and no deleterious effects were noticed. It seems quite probable, therefore, that they can be used effectively for controlling stripe. The results with the dusts indicate that the organism causing stripe probably is often under the hull of the barley and not inside of the seed coats. This possibility is strengthened by the fact that Johnson⁴ showed that barley became infected readily if the de-hulled seed was inoculated. While more experiments must be made, and yield data taken, it seems probable that chemical dusts eventually will be used successfully for the control of stripe.

SUMMARY

1. Four liquid fungicides, used at different temperatures and periods of soak, and ten dust fungicides were tested for their effectiveness in controlling barley stripe.

⁴ Johnson, *loc. cit.*

2. The effectiveness of the liquid fungicides depends on the temperature of the solution and the period of soak. Uspulun and Semesan were the most satisfactory fungicides for the control of stripe when the seed was soaked for one hour at room temperature. Uspulun, Germisan, and Semesan were about equally effective when the seed was soaked at 45° C. for one-half hour.

3. Yields were increased somewhat as a result of treating seed with the organic mercury compounds, although hot Germisan caused a delay in heading. Hot formaldehyde reduced yields in 1925, and also caused a delay in heading.

4. K-I-A and K-I-B dusts eliminated stripe entirely, while Du Pont dust No. 12, S. A. F. dust No. 225, and Wa Wa Dust were almost as effective. Coppercarb and Kolo Dust were ineffective in controlling stripe.

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CLUB ROOT IN RELATION TO SOIL ALKALINITY

CHARLES CHUPP

The effect of alkalinity on the presence of *Plasmodiophora brassicae* Wor. in the soil was noted even before the organism was described and named. It was found that an application of lime or wood ashes decreased the amount of disease, or controlled it entirely. This was before the time when apparatus was available for determining fairly accurately the acidity of the soil; so many workers merely guessed at the amount of lime to use, as is shown by some of the following recommendations:

256 bu. lime or 10-12 cu. yds. turf ashes an acre; Farquharson (3), 1831
14-16 tons lime an acre; Hunter (7), 1857
700 lbs. lime an acre (in drill with seed); Somerville (12), 1894
2 tons lime an acre; Voelcker (14), 1894
90 bushels lime an acre; Stewart (13), 1895
37.5-150 bushels lime an acre; Halsted (4), 1896
400 liters lime an acre; Mahieu-Sanson (9), 1897
6-8 tons lime an acre; Hawk (5), 1898
75-150 bushels lime an acre; Jones (8), 1898

Later writers, probably quoting Ravn (11), stated that the critical point at which the organism ceased to cause infection was at or slightly above the neutral point, which probably compared roughly with our present day designation of pH 7.00.

Only one writer seems to have made hydrogen-ion determinations of soils that were free of club-root, and of those in which it was abundant. Atkins (1) in Ireland found a field divided by a small stream of water. On one side of the stream, where the soil tested pH 6.6, *Plasmodiophora* caused much injury. On the other side there was no disease and the soil tested pH 6.7 or slightly above. Atkins realized that the information was not sufficient to justify drawing definite conclusions. Judging from the results of other workers, however, he felt that the above figures were not far wrong.

The writer undertook experiments to determine, if possible, the pH range in which the pathogene lives and is able to cause infection. The purpose of these tests was to determine the amount of hydrated lime required to adjust the pH values of the soil to a point at which the development of the club root organism would be inhibited. It soon was found, however, that the pH values increased rapidly after lime was added but then gradu-

ally diminished, so that the plants in any one series were subjected to a relatively wide range of hydrogen-ion concentration. It was found, also, that the reaction of the soil in pot cultures in the greenhouse fluctuated even more widely than did that of soil in the field.

The only method left for determining the relationship of club-root and soil alkalinity was to conduct numerous experiments in which the determinations were made at frequent intervals, and then plot a curve from all the averages thus procured. For the experiments, seven plots were staked off in the field with buffer plots at each end. The second and fifth plots were checks; the rest were treated with hydrated lime or with sulfur, at the following rates: Plot 1, 1,000 lbs. lime an acre; plot 3, 2,000 lbs. lime an acre; plot 4, 640 lbs. sulfur an acre; plot 6, 3,000 lbs. lime an acre; plot 7, 320 lbs. sulfur an acre. During each planting approximately 1,600 cabbage plants were grown in each plot.

For the inoculum, clubbed roots were ground in a meat grinder, and the watery pulp scattered along the furrow in which the seeds were dropped. When the plants were large enough so that club-root could be detected, half

TABLE 1.—*The effect of applications of lime and sulfur on the pH of the soil and the development of club-root on cabbage. Field experiments at New Brunswick, New Jersey*

Plot no.	Treatment	Percentage disease, July 6	pH, July 6	Percentage disease, July 26	pH, July 26	Percentage disease, Aug. 31	pH, Aug. 31
1	1000 lbs. lime an acre	30.8 \pm 3.47	5.78	71.0 \pm 3.5	5.93	85.7 \pm 0.96	6.22
2	Check—no treatment	40.9 \pm 4.7	5.20	89.0 \pm 2.18	5.20	93.32 \pm 3.44	$\left\{ \begin{array}{l} 5.51 \\ 6.08 \end{array} \right.$
3	2000 lbs. lime an acre	31.5 \pm 4.13	6.07	72.2 \pm 4.98	$\left\{ \begin{array}{l} 6.35 \\ 6.52 \end{array} \right.$	81.22 \pm 4.05	$\left\{ \begin{array}{l} 6.56 \\ 6.85 \end{array} \right.$
4	640 lbs. sulfur an acre	28.0 \pm 4.54	5.12	48.9 \pm 0.22	5.14	80.0 few plants alive	$\left\{ \begin{array}{l} 4.37 \\ 4.94 \end{array} \right.$
5	Check—no treatment	43.7 \pm 4.82	5.48	83.1 \pm 2.43	5.56	93.78 \pm 1.93	$\left\{ \begin{array}{l} 5.29 \\ 5.29 \end{array} \right.$
6	3000 lbs. lime an acre	28.8 \pm 4.01	6.61	42.5 \pm 6.59	6.68	3.2 \pm 0.78	$\left\{ \begin{array}{l} 7.09 \\ 7.24 \\ 7.36 \end{array} \right.$
7	320 lbs. sulfur an acre	28.6 \pm 6.53	5.04	45.0 \pm 6.92	4.83	91.0 few plants alive	$\left\{ \begin{array}{l} 5.44 \\ 5.70 \end{array} \right.$

of the plants were pulled up and the percentage of disease determined. The remaining plants, now having more space in which to grow, were left 20 days longer. The second crop was planted as soon as harvesting of the first was completed. Inoculum was again strewn in the furrow. The results of the two plantings are given in table 1.

The first determinations of the pH values were made by the colorimetric method, and are not so accurate as are later ones made with the potentiometer.

It can readily be seen from the table that 1,000 and 2,000 lbs. of hydrated lime an acre on soil with a pH only slightly above 5.00 had very little effect on the percentage of club-root. But when 3,000 lbs. were applied two months before planting so that the soil was slightly above pH 7.00, the amount of disease was reduced to 3.2 per cent.

In the greenhouse, where similar work was conducted by using inoculated soil in two-gallon earthen jars the percentage of disease in soil that attained pH 6.20 to 6.85 was never so high as it was in the field; but in other ways the results were quite comparable. This was true especially of the more alkaline soil.

Twenty-two jars were used, and six successive crops were grown. An attempt was made to have a fairly uniform gradation of soil reaction in each experiment, from soil too alkaline for the growth of the slime mold to that in which club-root developed at a maximum rate. Hydrogen-ion determinations were made by means of a potentiometer each week from the time the seed was sown until the plants were harvested. The results of these tests are summarized in table 2.

The results are shown more plainly in graph 1.

Apparently the point at which the growth of *Plasmodiophora brassicae*

TABLE 2.—*The effect of hydrogen-ion concentration of soil on the development of club-root of cabbage. Greenhouse experiments at New Brunswick, New Jersey*

No. of individual jars	Range of disease percentages	Average percentage disease	Minimum pH	Maximum pH	Average pH
14	0	0	7.52	8.00	7.71
4	Less than 2	1.2	6.65	7.33	7.30
24	2 to 10	3.8	6.20	6.95	6.61
14	11 to 20	15.6	5.80	6.62	6.20
4	21 to 30	25.6	5.89	6.46	6.20
6	31 to 40	32.5	5.72	6.41	6.05
10	41 to 50	44.5	5.47	5.94	5.71
12	51 to 100	84.6	5.46	6.00	5.73

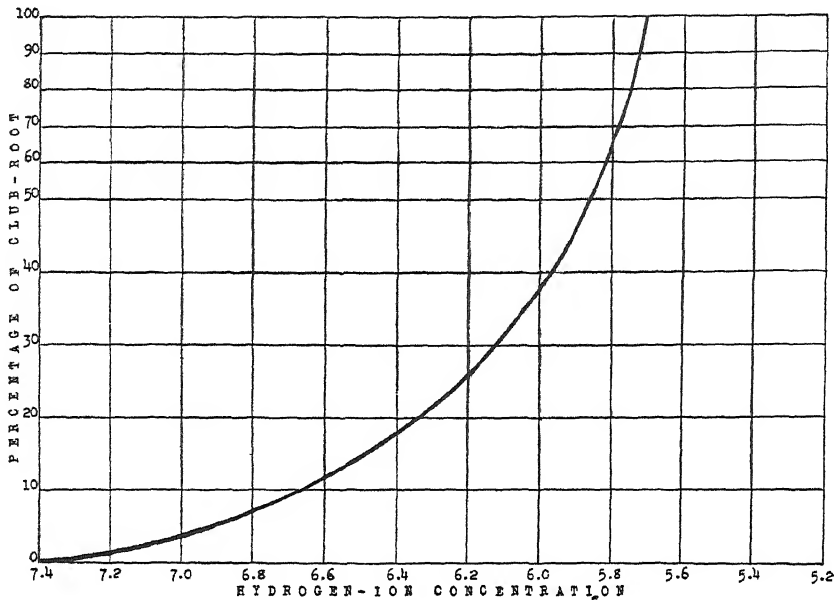


FIG. 1. The effect of soil acidity on the percentage of club-root.

is inhibited completely is not very definite. A trace of the disease was obtained on plants in greenhouse soil ranging from pH 7.2 to 7.4. Only one diseased root was ever found in soil that tested higher than this. Between pH 7.2 and 6.0 there was a rapidly increasing amount of club-root, until at a point below pH 6.0 it was possible to get almost 100 per cent of infection (figure 1). In fact, in a few cases both in the greenhouse and outdoors there was over 80 per cent of disease in soil that tested pH 6.6. These variations apparently were not due to different types of soil, for there seemed little or no difference in the use of dark loam sent from New York State as compared with the sandy clay of New Jersey.

Previous workers have raised the question as to whether the lime acts as a fungicide or merely retards the growth of the fungus. Ravn (11) did not believe it killed the spores, because quick-lime was no more effective in the production of a healthy crop of crucifers than was hydrated lime. The variation in hydrogen-ions of the soil in the jars used in the present experiment gave an opportunity for making further observations on this point. It was found that, when lime was added so that the soil tested considerably higher than neutral, no infection of the cabbage roots occurred. But when successive crops were grown in the same jar, the acidity of the soil finally

became high enough to permit infection of a large percentage of roots, even though no additional inoculum was added.

Hollrung (6) states that sulfur has been recommended for the control of club-root but adds that he does not know how effective it is. Müller-Thürgau and Osterwalder (10) attempted to control the disease by using a 1 to 3 mixture of sulfur and lime. In some cases it was broadcast, while in others it was mixed with the soil in the holes where the cabbages were to be set; in either case there was little or no control of the disease. As a result of using hydrated lime or stone lime, however, 80 to 86 per cent of the plants were clean. Collinge (2) gave as his opinion that a fall dressing of lime (1,500 lbs. an acre) followed by a spring dressing of sulfur (500 lbs. an acre) was better than lime alone.

In the field work, sulfur was compared with lime. In one plot 320 lbs. an acre of inoculated sulfur were raked into the top layer of soil before the cabbage was planted. In another plot 640 lbs. were applied.

TABLE 3.—*The effect of applications of sulfur on the development of club-root of cabbage. Field experiments at New Brunswick, New Jersey*

Soil treatment	First planting		Second planting		Third planting	
	No. plants	Percentage disease	No. plants	Percentage disease	No. plants	Percentage disease
Check	348	43.7	526	83.1	366	93.8
320 lbs. sulfur per acre	301	28.6	466	45.0	110	91.0
640 lbs. sulfur per acre	332	28.0	506	48.9	23	80.0
3000 lbs. lime per acre	261	28.8	401	42.5	425	3.2

The results of this test, presented in table 3, show that at first club-root was reduced slightly, and that the plants did not seem to be injured by the inoculated sulfur. But the third planting showed how sensitive cabbage is to sulfur injury. In addition, when sulfur was applied in the larger quantity, there seemed to be only a slight decrease in the amount of disease. Consequently this fungicide does not appear to be of value in the control of club-root.

SUMMARY

Investigators have long recognized that lime used in large quantities reduced the amount of club-root.

The present work shows that, under the conditions described above, pH 7.2 to 7.4 is the upper limit at which *Plasmodiophora brassicae* Wor. causes infection. The amount of disease increases very rapidly with an

increase in soil acidity. At pH 6.0 or even above, 100 per cent infection is possible.

Sulfur injures the cabbage plant and does not control club-root.

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VARIETAL SUSCEPTIBILITY OF POTATOES TO FUSARIUM WILT AND STEM-END ROT¹

R. W. Goss

Observations made during the past seven years indicate that there is considerable variation in the susceptibility of the potato varieties grown in Nebraska, to Fusarium wilt and stem-end rot previously described by the author.² It has not been uncommon to find fields of Irish Cobblers with a much higher percentage of wilt than Bliss Triumphs planted in the same field and growing under similar conditions. A careful check of the seed used in a number of instances indicated that the infection was chiefly from the soil and that the differences in the amount of infection might possibly be explained on the basis of varietal susceptibility.

In the winter of 1925-26, a preliminary test was made in the greenhouse. Healthy potatoes of the Triumph and Cobbler varieties were planted in soil that had been sterilized and subsequently inoculated with pure cultures of *Fusarium eumartii*. While only 30 plants of each variety were used, the differences in the amount of infection were very marked. The Triumph plants showed 30 per cent slight wilt; while 90 per cent of the Cobblers were wilted, and most of the wilt was of a severe type, resulting in the death of the plants. It was found, by examination of the underground portion of the plants, that 80 per cent of the Triumphs had slightly infected roots and 100 per cent of the Cobblers were severely infected. The stems of 70 per cent of the Triumphs were healthy, while all of the stems of the Cobblers showed some discoloration. The high prevalence of root infection in the Triumphs, together with the lightness of the infection as indicated by the lack of any severe wilt, indicated that this variety did have some degree of resistance to the disease as compared with the Cobblers.

In 1926 a test was conducted in the field by planting healthy Irish Cobblers and Bliss Triumphs in alternate rows on land that had produced a crop of potatoes the previous year with a fairly high percentage of wilt. One half of the seed potatoes of each variety was treated with a 1-10 solution of Semesan Bel after cutting. This was done to determine whether an organic mercury disinfectant used in this way would prevent infection of the seed piece from the soil.

¹ Published with the approval of the Director as Paper No. 47, Journal Series, of the Nebraska Agricultural Experiment Station, Lincoln, Nebr.

² Goss, R. W., Potato wilt and stem-end rot caused by *Fusarium eumartii*. Neb. Agr. Exp. Sta. Res. Bul. 27. 1924.

In 1927 a similar test was conducted in which Early Ohio potatoes were included. Half of each variety was again treated with Semesan Bel, and the untreated and treated seed pieces were planted in alternate, 150-hill rows on land presumably infested with *Fusarium eumartii*.

Both in 1926 and 1927 the climatic conditions were unfavorable for the development of wilt in the field: fairly low temperatures with considerable precipitation prevented the development of severe symptoms. When the potatoes were dug and examined it was found that some infection had occurred even though no foliage symptoms had appeared. Every tuber was cut and examined for stem-end rot and vascular discoloration. The results are presented in table 1. Most of the infection occurred as a vas-

TABLE 1.—*The effect of seed treatment on percentage of wilt infection in three varieties of potato. Based upon the weight of infected tubers*

Variety	Treatment	Percentage of wilt ^a	
		1926	1927
Triumph	None	15.6	5.7
do	Semesan Bel	9.6	9.7
Cobbler	None	28.9	10.6
do	Semesan Bel	24.7	12.1
Early Ohio	None	—	13.1
do	Semesan Bel	—	13.3

^a The percentages are based upon the total weight of potatoes from 300 hills for each treatment of Triumphs and Cobblers in 1926 and 450 in 1927. Only 300 hills of Early Ohio were used for each treatment.

cular discoloration, typically a band of soft brown tissue from one-eighth to one-fourth of an inch wide, extending in a few cases through the entire tuber. Quite often this vascular discoloration was associated with a stem-end rot, which was characterized by sunken areas and from which *Fusarium eumartii* was isolated.

The results presented in table 1 show that the Irish Cobbler and Early Ohio varieties are equally susceptible and that they are both much more susceptible than Bliss Triumph. In the untreated rows of each variety only about half as much infection occurred in the Triumphs as in the other two varieties. The difference between these varieties appears to be chiefly in degree of susceptibility. The writer does not intend to convey the impression that the Triumph does not become severely infected with wilt. Many fields of Triumphs have been observed with from 30 to 50 per cent of wilt infection, and the disease in this variety is one of the worst with which the grower has to contend in western Nebraska. The greater sus-

ceptibility of the other varieties, however, means that they should never be planted on heavily infested soils.

The results with seed treatments are not so clear cut. In 1926 there was some decrease in infection when seed was treated with Semesan Bel, but in 1927 the treatment apparently had no beneficial effect and there was even a slight increase in the amount of disease. Either the disinfectant is of no great value in protecting the seed piece from infection or else infection takes place through the roots. Root infection is considered to be the more common method of invasion in Nebraska.

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PHYTOPATHOLOGICAL NOTES

*A "Streak" of Tomatoes Produced by a Disturbing Principle from Apparently Healthy Potatoes in Combination with Tomato Mosaic Virus.*¹—

An investigation of the nature of a severe mosaic disease of the tomato prevalent in the commercial tomato fields of Utah has identified the disease as "streak" of tomatoes. This disease has been shown by T. C. Vanterpool² to be produced by the combination of tomato mosaic and potato mosaic viruses in the infected tomato plant. During the course of the investigations evidence has been obtained to the effect that apparently healthy potatoes of certain commercial varieties carry some disturbing principle which, when in combination with tomato mosaic virus, is capable of producing the same set of symptoms as characterize streak.

Green Mountain, Early Ohio, and Irish Cobbler potatoes have been used as sources of inoculum for inoculation experiments with healthy potato juices. The Green Mountain potatoes were sent by Dr. E. S. Schultz, of the United States Department of Agriculture, to the Department of Plant Pathology of the Utah Agricultural Experiment Station for the purpose of conducting mosaic-disease investigations. The Early Ohio potatoes were grown on the Station potato seed plots and indexed in the Station greenhouse. The Irish Cobbler potatoes were sent by Dr. M. B. McKay, of the United States Department of Agriculture (located at Corvallis, Oregon), for work on the new disease of potatoes prevalent in Utah this last summer (1927).

The Green Mountain and the Early Ohio tubers producing the vines from which the juices were obtained for the inoculation experiments were indexed in the Station greenhouse in the early spring of 1926. From appearance they seemed to be free from all visible virus troubles. The Irish Cobbler tuber was cut in half and both halves planted in the greenhouse under celluloid cages. Both halves produced apparently healthy plants. So far as could be observed, all the plants from which juices were obtained were perfectly healthy and normal. Juices from these potato plants inoculated into healthy tomato plants (inoculated at the same time with tomato-mosaic virus) produced symptoms of "streak" as severe as, and—under the conditions observed—indistinguishable from, those of the "streak" produced by the combination of tomato-mosaic and potato-mosaic viruses on the tomato. Further investigations are in progress at this Station.—H. L. BLOOD, Department of Plant Pathology, Utah Agricultural Experiment Station, Logan, Utah.

¹ Approved for publication by the Director, December 22, 1927.

² Vanterpool, T. C. Streak or winter blight of tomato in Quebec. *Phytopath.* 16: 311-331. 1926.

Photographic collection of Erwin F. Smith. The photographic collection of the late Dr. Erwin F. Smith has, through the generosity of his colleagues, been placed in the hands of Science Service. This consists of about 200 negatives, together with a large number of prints. These are mostly of persons now active in phytopathology, though the collection also contains many portraits of notable figures in the history of botany.

The collection is now being arranged and catalogued. After they have been properly labeled, all the portraits, together with a complete set of prints from the negatives, will be deposited with the Library of the U. S. Department of Agriculture. The negatives will be retained by Science Service. A special list of these will be issued in the near future, so that phytopathologists and botanists generally may have an opportunity to secure photographs of their fellow-workers.

Correction. Correction for "Virus mixtures that may not be detected in young tobacco plants" by H. H. McKinney, *Phytopath.* **16**: 893.—The following mistake occurs in my paper, reference to which is given above:

line 8, *read* 32.5°–37.5° C. *for* 90°–100° C.

Inasmuch as the list of errata for volume 16 already has been published, I am taking this means of calling attention to the error.—H. H. McKINNEY, U. S. Department of Agriculture.

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COMPARATIVE STUDIES OF CERTAIN CLADOSPORIUM DISEASES OF STONE FRUITS.

M. BENSAUDE AND G. W. KEITT

In consequence of its wide distribution and of the serious losses which it occasions, peach scab (caused by *Cladosporium carpophilum* Thüm.) has been the subject of numerous investigations. Similar diseases of other stone fruits have long been observed, but have received less study. Doubt still exists, therefore, concerning the interrelationships of the *Cladosporium* diseases of the various stone fruits and of the organisms which cause them. During a sojourn of the senior author at the University of Wisconsin, the present writers undertook some comparisons of those *Cladosporium* diseases of stone fruits of which suitable material was available (See preliminary report by Bensaude and Keitt, 2). A brief account of this work¹ follows.

SYMPTOMS

The symptoms of peach scab are well known, having been described and illustrated by many writers (See Keitt, 3, p. 6-8, Pls. I-IV). The previous accounts of the *Cladosporium* diseases of plum, apricot, and cherry, however, have been somewhat less complete, and are, therefore, supplemented by the following descriptions.

Plum scab

On fruit.—At Madison, Wis., in 1922 the first macroscopic evidence of scab was observed on fruits of *Prunus americana* Marsh. about June 15, when the young plums were approximately 8 millimeters in diameter. The typical lesion first appeared as a small, circular, olivaceous patch, the color of which was due chiefly to the fruiting of the causal fungus. Very soon,

¹ Publication of these results, which were limited in scope by the time available, has been delayed in the hope that the junior author might supplement them by further studies. As this has not yet been feasible, it seems desirable to report the available results without further delay.

however, the epidermal and hypodermal host cells under the olivaceous area and in a narrow zone about it developed a yellowish color. Later, as suberization progressed, the central part of the lesion took on a brown, corky aspect. As the fruit began to color, a red halo commonly appeared around the lesion. On the dark carmine, ripe fruit the lesions were much like the surrounding surface in color, except for lack of gloss and for the olivaceous or brown aspect of the fungus and of the suberized area. At this stage the spots frequently had a slightly raised border, and were rather sharply defined. In the later stages of the development of the lesions, the fungus commonly continued to grow in its subcuticular position, forming a pseudo-parenchymatous structure from several to many cells in thickness. In the period of rapid fruit growth prior to ripening, the lesions in which cork development had been most marked commonly cracked. At any stage after the lesions became macroscopic, conidiophores and conidia could be found upon the surface.

Lesions were found to occur commonly on the pedicels. Before they became macroscopically evident, the conidial fructification of the fungus could be detected by use of a hand lens. As the growth of the fungus became more dense, it gave a grayish olivaceous aspect to the diseased area. Later, as the pedicels became yellowish, the lesions became suberized and brown, and were clearly delimited. Conidiophores grew abundantly on these lesions, and commonly bore an abundant crop of conidia when held over night in a moist chamber. Sometimes old lesions on the pedicels sloughed off late in the season.

On twigs.—The lesions on twigs were of the same general type as those on peach twigs (Keitt, 3, p. 7), except that they were commonly smaller, probably due to the fact that the wild plum twigs examined had made a much less vigorous growth than those of well cultivated peach trees.

On leaves.—The leaves of *P. americana* did not appear to be very susceptible to injury by *Cladosporium*. The symptoms on the leaves were not conspicuous. The chief macroscopic evidence of infection was a slight brown or olivaceous discoloration, which usually occurred on the midribs or veins on the lower (dorsal) surface. Conidiophores and conidia were found on these discolored areas. Infection occurred quite commonly on the petioles, where the lesions much resembled those which develop on the pedicels.

Apricot scab

The only lesions on *Prunus armeniaca* L. available for examination were those induced on twigs and leaves by inoculation.

On twigs.—The lesions first appeared as reddish flecks, which later became brown. Their development closely paralleled that of lesions on twigs of peach and plum.

On leaves.—Infection was always more abundant on the lower (dorsal) surface, though conidiophores and conidia have been observed to occur sparsely on the upper surface. The very young lesions were commonly angular in outline, conforming with the areas bounded by the smallest veinlets. At first pale, olivaceous yellow, they commonly became more yellow with age. On the parts of the leaf more exposed to the sun they often took on a bright red or purplish tint. The lesions were found to develop on the lamina or on the veins, midrib, or petiole. When they occurred in sufficient abundance, they frequently became confluent. When fully developed they ordinarily varied from one to several millimeters in diameter.

Cherry scab

Scab was observed on unsprayed, neglected Early Richmond trees (*Prunus cerasus* L.) at Sturgeon Bay, Wis.

On fruit.—The first evidence of fruit infection was observed as barely macroscopic pink splotches, which had a stippled appearance under the hand lens because of unequal reddening of the sap of the cells of the affected areas. As the lesions, which were unchanged in elevation and imperfectly demarked from the surrounding tissue, enlarged slowly, the color of the middle area faded to a dull red or brown, while the brighter red continued to be developed in the advancing peripheral zone, which was usually about one-half millimeter wide, and sharply defined at the inner margin. The coloration of the lesions varied somewhat with the stage of development of the fruit. The fully developed lesions varied from one-half to two millimeters in diameter. They showed no change in elevation or hardening of tissue. They usually bore conidia of the causal fungus rather sparsely when freshly collected from nature, but in abundance after being held over night in a moist chamber. As many as 40 lesions were found on a single badly diseased fruit.

On twigs.—The first macroscopic evidence of twig infection appeared as small, irregular to oval, imperfectly defined, brownish areas about one-half millimeter in greatest dimension. The color at this stage is due chiefly to the presence of conidiophores and conidia of the causal fungus. The fully developed lesions were usually irregular to oval, and little changed in general aspect, though they sometimes took on a brown color and became very slightly elevated.

On leaves.—The lesions first appeared on the upper (ventral) surface of green leaves as barely visible flecks, slightly lighter than the normal green. At this stage it was necessary to secure very favorable illumination in order to see them. They were irregular to circular in outline, unchanged in elevation, and bore abundant conidia of the causal fungus. No

lesions larger than one to two millimeters in diameter were observed. The amount of material examined, however, was limited. On leaves which were yellowing, the infected areas and a peripheral zone commonly held their green color longer than did the surrounding tissue. No lesions were observed on the under (dorsal) surface of the leaves.

MODE OF PENETRATION OF THE HOST BY *CLADOSPORIUM CARPOPHILUM*

In preliminary experiments it was found that the leaves of apricot are very susceptible to infection by *C. carpophilum* and that potted apricot plants thrive well under greenhouse conditions. Consequently, this host (variety Superba) was chosen for studies of the mode of penetration of leaves by the fungus.

The inoculum consisted of a suspension in sterile distilled water of spores from single-spore cultures of a strain of *Cladosporium* which had been isolated at Madison, Wis., from *P. americana* and grown on Lima bean agar. The cultures used were fresh (usually 8 to 12 days after transfer) and bore abundant conidia. The viability of the spores was tested by placing drop-lets of the inoculum on clean glass slides which were incubated in a moist chamber at approximately 22° C. Ordinarily, from 80 to 100 per cent germinated within 48 hours.

Drops of the inoculum were placed on marked areas on the lower (dorsal) surface of the leaves. They were permitted to dry, and other drops were added. The potted plants were then placed in a large moist chamber in which the temperature was approximately 22° C. and the relative humidity was approximately 100 per cent. After three or four days the plants were placed either in a well-illuminated chamber in which the temperature was approximately 22° C. and the relative humidity 85 per cent, or out of doors. On the second day and at intervals thereafter, leaves were collected and examined. To facilitate examination the following method was used. The inoculated areas were placed for 6 to 12 hours in a solution consisting of equal parts by volume of glacial acetic acid and 95 per cent alcohol. They were then cleared for one to several days in a saturated aqueous solution of chloral hydrate. They were then immersed for 6 to 12 hours in Pianeze's stain (Vaughan, 6), after which they were cleared in carbol turpentine for a few hours. In successful preparations the spores or germ tubes on the surface of the leaves were stained purple or blue, whereas the subcuticular mycelium was colored in magenta or pink. The epidermal cells of the leaves were stained a bright green. If a permanent mount was desired, the leaf fragments were rapidly dehydrated, washed in clove oil and xylol, and mounted in Canada balsam. After this treatment, the spores and surface mycelium were red instead of purple, while the other

parts which have been mentioned were not considerably changed in color if immersion in alcohol was not prolonged.

Examinations made on the second day after inoculation commonly showed that a high percentage of the spores had germinated. The germinating spores produced from one to three germ tubes each. Some of these tubes were comparatively long. These failed to develop infection hyphae. The tubes from which penetration was observed to occur were comparatively short, measuring from 7 to 35 microns in length. With the technique used, however, no penetration was observed in material collected on the second day. The material of the second collection, which was made four days after inoculation, showed abundant infection. Sufficient subcuticular development had occurred to suggest that penetration had taken place considerably before this collection was made. From field observations and from experience with other closely related parasites it seems probable that penetration occurs in a much shorter time than four days.

In the material studied penetrating hyphae were observed in large numbers. No well-defined appressoria were seen. Penetration occurred at or near the apex of the germ tube, the circular lumen of the penetrating hypha being visible as a delicate circular orifice (Figs. 1, 2). A slightly lower focus often revealed the subcuticular mycelium. In the type of preparation studied the detailed structure of this mycelium was not sharply defined. In the early stages following penetration, it often appeared as a small, some-

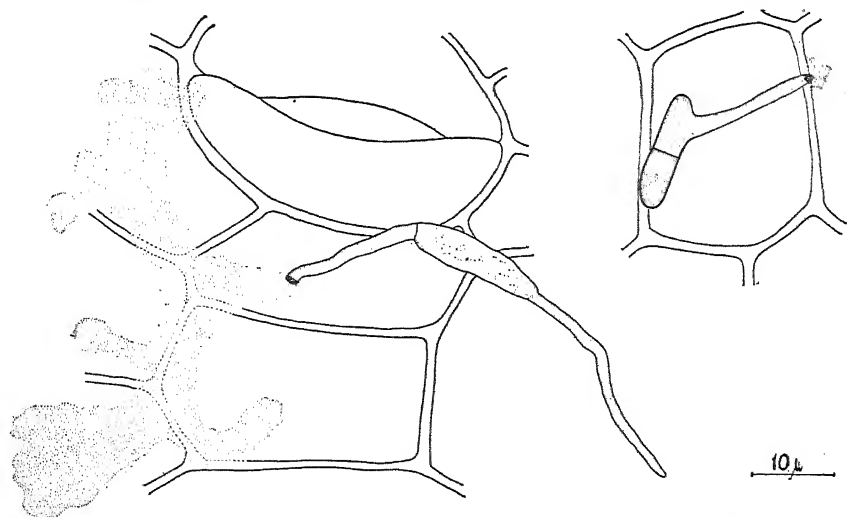


FIG. 1. *Cladosporium* from *P. americana* parasitizing the dorsal surface of a leaf of *P. armeniaca*, four days after inoculation with conidia. Camera lucida drawing of material stained *in toto*. The stippled strands show the general distribution of the subcuticular mycelium.

what amoeboid rosette about the point of entry of the penetrating hypha. With further growth, flattened ribbon-like strands of subcuticular mycelium developed, frequently seeming to follow roughly the outlines of the epidermal cells. As has been reported for various other parasitic fungi, penetration appeared to occur more frequently near the boundaries of epidermal cells than over the middle portions of these cells. At the time when the lesions first became macroscopically evident, these flattened ribbon-like strands of subcuticular mycelium had ramified the invaded areas. Conidiophores were borne profusely from this mycelium, penetrating the cuticle freely, whether they were developed near the boundaries of epidermal cells or over the middle portions.

A more detailed record of the processes of infection should be revealed by studies of sections prepared by the usual histological methods. Such work was contemplated, but could not be completed in the time available.

CROSS-INOCULATION EXPERIMENTS

The inoculum used consisted of suspensions in sterile distilled water of spores and mycelial fragments taken either from sporulating single-spore

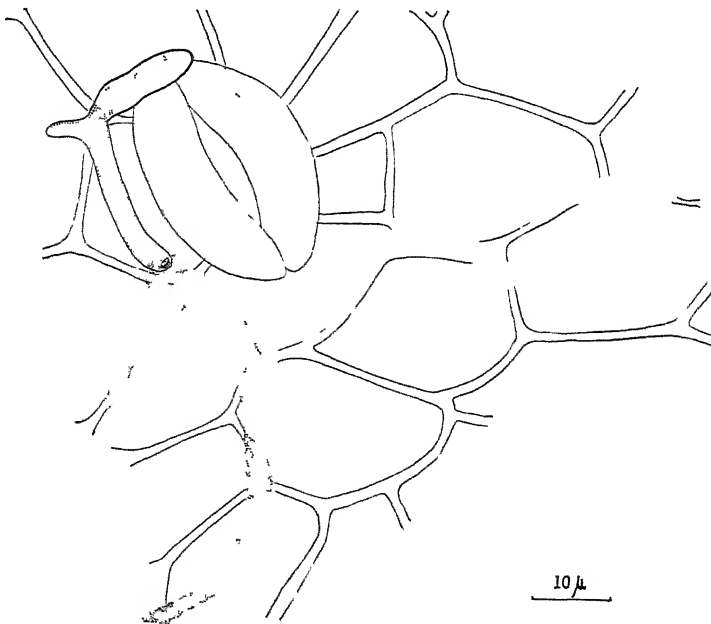


FIG. 2. *Cladosporium* from *P. americana* parasitizing the dorsal surface of a leaf of *P. armeniaca*, nine days after inoculation with conidia. Camera lucida drawing of material stained *in toto*.

pure cultures on Lima bean agar or from sporulating lesions on leaves or fruits of naturally infected *Amygdalus persica* L., *P. americana*, or *P. cerasus*. The species inoculated were *A. persica* (Summer Heath), *P. armeniaca* (Superba), *P. americana* (seedlings of native wild plum), *P. cerasus* (Montmorency and Early Richmond), and *P. domestica* L. (Lombard). In some tests the plants were in pots in the greenhouse; in others, they were in plantings out of doors.

The inoculum was applied to marked twigs and to both surfaces of the leaves by means of sterile atomizers. After inoculation the potted plants were placed in a large moist chamber at temperatures of 18 to 23° C. for three days and then on a greenhouse bench or out of doors, depending upon the season. Plants growing out of doors were inoculated in a similar manner and kept moist for three days by the apparatus described by Keitt (4, p. 546-547). An appropriate number of uninoculated plants of each species studied served as controls. The experimental plants were examined and noted at suitable intervals. The results appear in table 1.

The *Cladosporium* from *A. persica* induced abundant infection on the leaves and twigs of *A. persica* and *P. armeniaca*, and slight infection on the leaves of *P. americana*. No evidence of infection appeared upon *P. cerasus* or *P. domestica* inoculated with the fungus from the peach.

The fungus from *P. americana* induced infection upon the leaves of *P. americana* and *A. persica* and the leaves and twigs of *P. armeniaca*. No evidence of infection appeared upon the plants of *P. domestica* and *P. cerasus* inoculated with the plum fungus.

The *Cladosporium* from *P. cerasus* induced infection on leaves of *P. cerasus* (Early Richmond). Flecks were several times observed on leaves of *P. armeniaca* which had been inoculated with the cherry fungus. However, no fructification of *Cladosporium* was ever found on these flecks. No lesions or flecks were observed to follow inoculation of the cherry *Cladosporium* upon *A. persica*. However, in one instance five or six conidiophores and a few conidia which appeared to be *Cladosporium* were found on one peach leaf thus inoculated. The identity and significance of these were not clearly established.

From these data it appears that strains of *Cladosporium* from *A. persica* and *P. americana* are pathogenic on *A. persica*, *P. americana*, and *P. armeniaca*. *P. cerasus*, however, appears to be highly resistant to these strains. The *Cladosporium* from *P. cerasus* induced no typical infection on any of the host plants on which it was tried, except *P. cerasus*.

None of the control plants showed any evidence of disease. To conserve space, the data from these plants are omitted from table 1.

Observations were made with the aim of determining whether failure to effect cross-inoculations in certain instances was due to inability of the

TABLE 1.—Summary of results of cross-inoculation experiments with *Cladosporium* spp. on stone fruits, Madison, Wis., 1921-1922

Location and date of inoculation	Inoculum		Plant inoculated	Minimal period of incubation (days)		Infection
	Host from which obtained	Source		On leaves	On twigs	
In greenhouse						
May 18, 1922	<i>Amgdalus persica</i>	19-day-old culture	<i>Amgdalus persica</i>	None
do	do	do	<i>Prunus armeniaca</i>	31	Slight
do	do	do	<i>P. cerasus</i> (E. R.)	None
July 21, 1922	do	11-day-old culture	<i>A. persica</i>	64	Abundant
do	do	do	<i>P. cerasus</i> (E. R.)	None
Out of doors						
July 19, 1921	do	20-day-old culture	<i>A. persica</i>	15	22	Abundant
do	do	do	<i>P. armeniaca</i>	16	30	do
do	do	do	<i>P. domestica</i>	None
do	do	do	<i>P. americana</i>	39	Slight
Aug. 10, 1921	do	14-day-old culture	<i>P. domestica</i>	None
do	do	do	<i>P. americana</i>	52	Slight
do	do	do	<i>P. cerasus</i> (E. R.)	None
July 21, 1922	do	do	<i>A. persica</i>	33	Abundant
do	do	do	<i>P. armeniaca</i>	31	do
In greenhouse						
May 25, 1922	<i>Prunus americana</i>	Culture	<i>A. persica</i>	None
do	do	do	<i>P. armeniaca</i>	24	28	Abundant
do	do	do	<i>P. cerasus</i> (E. R.)	None
June 1, 1922	do	do	<i>A. persica</i>	86	Fair
do	do	do	<i>P. armeniaca</i>	23	Abundant
do	do	do	<i>P. cerasus</i> (E. R.)	None
June 21, 1922	do	4-day-old culture	<i>P. armeniaca</i>	10	Abundant
June 24, 1922	do	Culture	do	13	do
July 8, 1922	do	10-day-old culture	do	30	Slight

TABLE 1.—(Continued)

Location and date of inoculation	Inoculum		Plant inoculated	Minimal period of incubation (days)		Infection
	Host from which obtained	Source		On leaves	On twigs	
In greenhouse	<i>P. armeniacum</i>					
July 13, 1922	do	12-day-old culture	<i>P. armeniacum</i>	27	Abundant
July 21, 1922	do	4-day-old culture	<i>A. persica</i>	None
do	do	do	<i>P. armeniacum</i>	62	Slight
do	do	do	<i>P. cerasus</i> (E. R.)	None
Out of doors						
Aug. 2, 1921	do	Lesions	<i>A. persica</i>	44	Slight
do	do	do	<i>P. armeniacum</i>	41	Abundant
do	do	do	<i>P. domestica</i>	None
do	do	do	<i>P. armeniacum</i>	None
June 19, 1922	do	16-day-old culture	<i>P. armeniacum</i>	25	Abundant
do	do	do	<i>P. armeniacum</i>	25	Slight
do	do	do	<i>P. armeniacum</i>	None
do	do	do	<i>P. cerasus</i> (E. R.)	None
June 29, 1922	do	9-day-old culture	<i>A. persica</i>	None
do	do	do	<i>P. armeniacum</i>	18	Slight
do	do	do	<i>P. cerasus</i>	None
In greenhouse						
April 27, 1922	<i>P. cerasus</i>	14-day-old culture	<i>A. persica</i>	None
do	do	do	<i>P. armeniacum</i>	None
do	do	do	<i>P. cerasus</i> (M.)	None
do	do	do	<i>P. cerasus</i> (E. R.)	58	Slight
May 9, 1922	do	10-day-old culture	<i>A. persica</i>	^a
do	do	do	<i>P. armeniacum</i>	None
do	do	do	<i>P. cerasus</i> (M.)	None
do	do	do	<i>P. cerasus</i> (E. R.)	15	Abundant
July 14, 1922	do	16-day-old culture	<i>P. armeniacum</i>	None
do	do	do	<i>P. cerasus</i> (E. R.)	26	Abundant

^a No macroscopic lesion was developed. Microscopic examination revealed one group of a few conidiophores and conidia which resembled *Cladosporium*.

fungus to penetrate the host or to other reasons. Application of the technique described earlier showed clearly that the cuticle of the leaves of *P. cerasus* was commonly penetrated by the *Cladosporium* from *P. americana*, though little development of the fungus occurred after penetration. The cuticle of leaves of *P. armeniaca* was similarly penetrated by the fungus from *P. cerasus*. Slight flecking of the leaves but no sporulation of the fungus followed this inoculation. These results are very similar to those reported by Wiltshire (6) for *Venturia inaequalis* (Cke.) Wint. and *V. pyrina* (Cke.) Aderhold on apple and pear.

RELATIONS OF TEMPERATURE

Conidia from pure cultures of *Cladosporium* from *P. americana* and *P. cerasus*, respectively, were suspended in sterile distilled water. Drops of these spore suspensions were placed on the surfaces of agar (2 per cent agar in water) in petri dishes, which were incubated in dark chambers at a range of constant temperatures (variable 1° C.). After 24 hours, data were taken on the percentage of spores germinated and, in certain series, on the length of germ tubes. The results are shown graphically in figures 3 and 4. The data shown in figure 3 are averaged from four series, in each of which the records were made from 50 spores chosen at random in each plate. The results shown in figure 4 were taken similarly from two series, being based

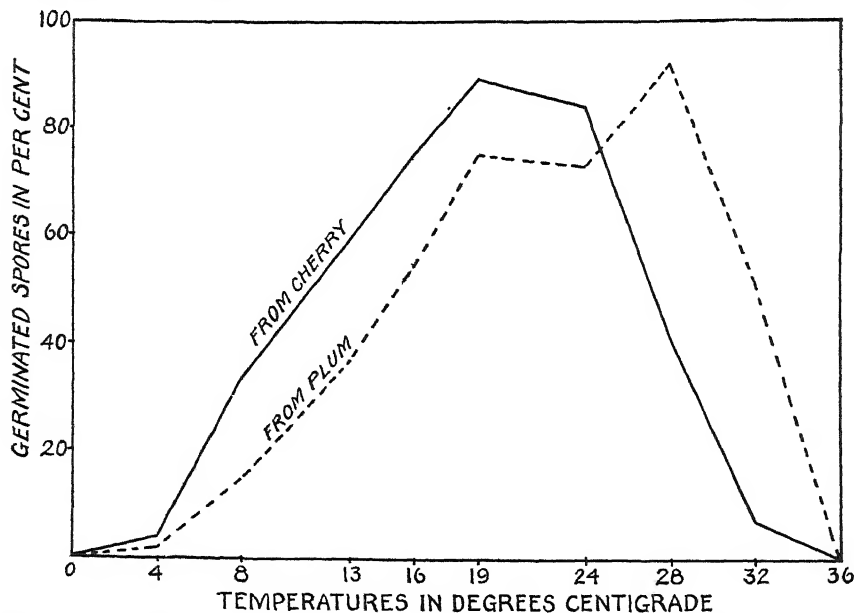


FIG. 3. The relation of temperature to the germination of conidia of *Cladosporium* from cherry (*P. cerasus*) and plum (*P. americana*).

on measurements of the length of the germ tubes of ten germinated spores in each plate. These data suggest that the *Cladosporium* from *P. americana* has a perceptibly higher range of temperature for conidial germination than that from *P. cerasus*. The records in figure 4 indicate that, under the conditions of these experiments, the germ tubes of the cherry fungus elongate more rapidly than do those of the plum *Cladosporium*.

Drops of spore suspensions of the type just described were placed on the surfaces of Lima bean agar in petri dishes which were then incubated at a range of constant temperatures (variable 1° C.). In each series it was sought to have the drops as nearly uniform as feasible in size and in the area of their distribution on the agar. After 10 days, observations were made on the macroscopic aspects of the fungal growth. No significant difference was observed in the development of strains of *Cladosporium* isolated, respectively, from *P. americana* and *A. persica*. With each of these strains, little macroscopic evidence of growth appeared at temperatures below 16° C. The most vigorous growth occurred within the range of 19–28° C. Good, but less vigorous, growth occurred at 31–33° C., the highest temperature tried. In so far as macroscopic appearance is a criterion of growth, the *Cladosporium* isolated from *P. cerasus* seemed to have a distinctly lower temperature range on the medium used. In this case, good growth oc-

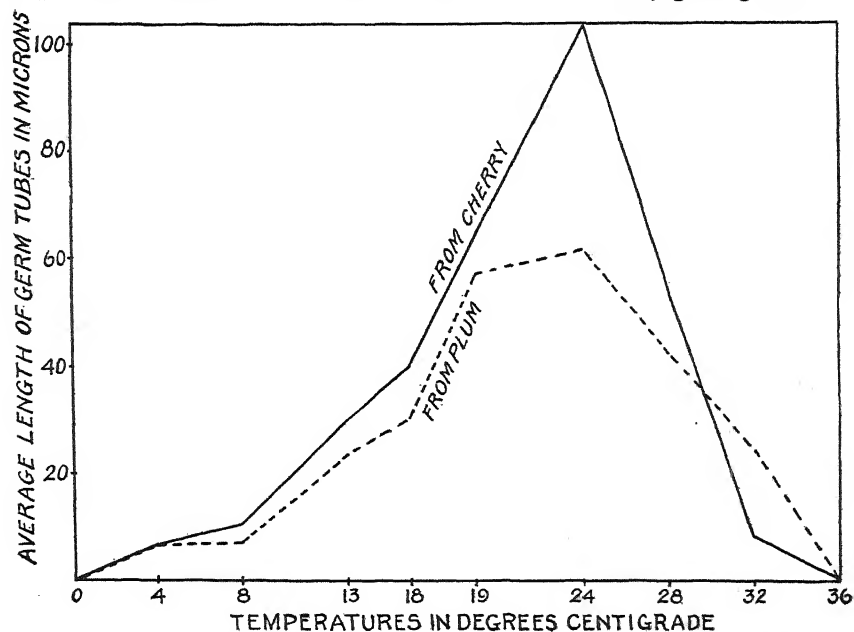


FIG. 4. The relation of temperature to the length of germ tubes developed by conidia of *Cladosporium* from cherry (*P. cerasus*) and plum (*P. americana*).

curred at 13–16° C. The most vigorous development was observed in the range of 19–26° C. At 28–31° C. growth was much retarded. At 31–33° C. no macroscopic evidence of growth was observed.

While these data are too limited to be fully conclusive, they strongly suggest that the *Cladosporium* which occurs on *P. cerasus* has a somewhat lower thermal range for germination and growth than have the strains which parasitize *P. americana* and *A. persica*.

MORPHOLOGY

Comparative studies of conidia and conidiophores revealed no sharply defined morphological differences in the *Cladosporium*s on *A. persica*, *P. americana*, and *P. cerasus*. The material studied conformed closely with the descriptions by von Thümen (4, p. 13), Aderhold (1, p. 542), and Keitt (3, p. 12–14). Measurements were made of the lengths of 300 conidia from lesions on fresh material from each of these hosts. The spores were mounted under a thin cover slip on a clear agar gel and measured promptly under an oil immersion lens. The results, which appear in figure 5, show a

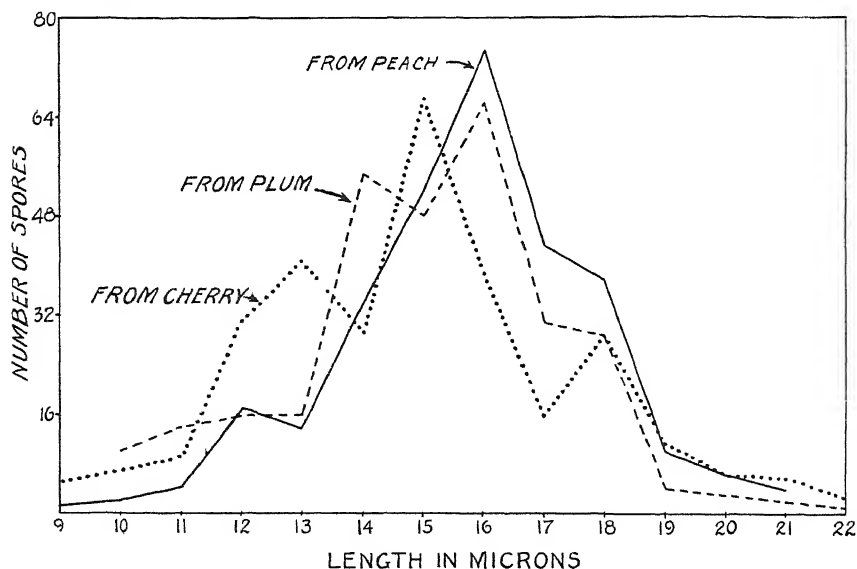


FIG. 5. A graphic summary of length measurements of conidia of *Cladosporium*s from field material.

close agreement in the measurements of the strains from *A. persica* and *P. americana*. The conidia from *P. cerasus* were slightly shorter than those from the other two hosts. The data, however, are not sufficiently extensive to indicate clearly whether or not this difference is significant. As is shown

in table 2, it was lessened when the conidia measured were taken from cultures grown according to a standardized method. Further studies will be necessary to determine whether or not the suggestions of bi- and trimodality in the polygons of the plum and cherry strains are significant. The conidia which were measured varied in width from 3.5 to 6 microns, the most common range extending from 4 to 5.5 microns.

TABLE 2.—*Length of conidia of Cladosporiums from certain stone fruits^a*

Source of conidia	Number measured	Average length in microns
Lesions on fruit of <i>Amygdalus persica</i>	100	16.3
Lesions on fruit of <i>Prunus americana</i>	100	16.2
Lesions on fruit of <i>P. cerasus</i>	100	15.6
Culture from <i>A. persica</i>	100	18.9
Culture from <i>P. americana</i>	100	19.1
Culture from <i>P. cerasus</i>	100	18.2

^a The measurements of spores from lesions were made in August from freshly collected specimens. The cultures from which measurements were made were grown comparatively on potato dextrose agar slants in a dark chamber at 23° C.

CULTURAL STUDIES

Strains of *Cladosporium* from *A. persica*, *P. americana*, and *P. cerasus*, respectively, were grown comparatively on several media, including Lima bean and potato dextrose agars. No significant differences were observed in the development of the peach and plum strains on these media. In the earlier stages of development of thalli from germinating spores, the *Cladosporium* from *P. cerasus* varied distinctly from the other two strains (Figs. 6, 7, and 8). The conidia of this strain showed little swelling during germination, and gave rise to comparatively long, slender germ tubes. In the stages of development represented in figures 7 and 8, the young thalli of the cherry fungus were distinctly less abundantly septate than those of the other two strains, the cells were less torulose, and the conidia from which the thalli developed maintained their identity. The young thalli of the strains from *A. persica* and *P. americana* were more abundantly septate and branched, while the swollen conidia soon lost their identity among the torulose cells which adjoined them. The thalli of these two strains tended to produce conidia earlier than did those of the cherry fungus. In the earlier macroscopic stages of development on the culture media used,

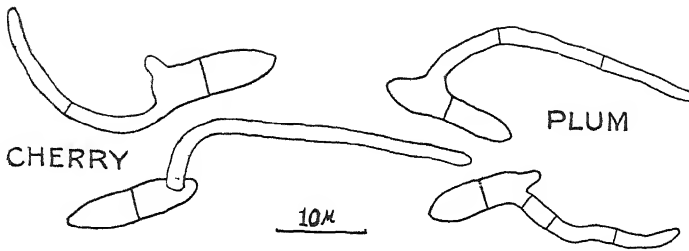


FIG. 6. Young thalli of *Cladosporium* from cherry (*P. cerasus*) and plum (*P. americana*) 36 hours after conidia were placed on plates of 2 per cent agar in water at 23° C. Traced from photomicrographs.

the cherry fungus grew somewhat less compactly than the others, and showed rather more aerial mycelium. This difference lessened as the cultures grew older.

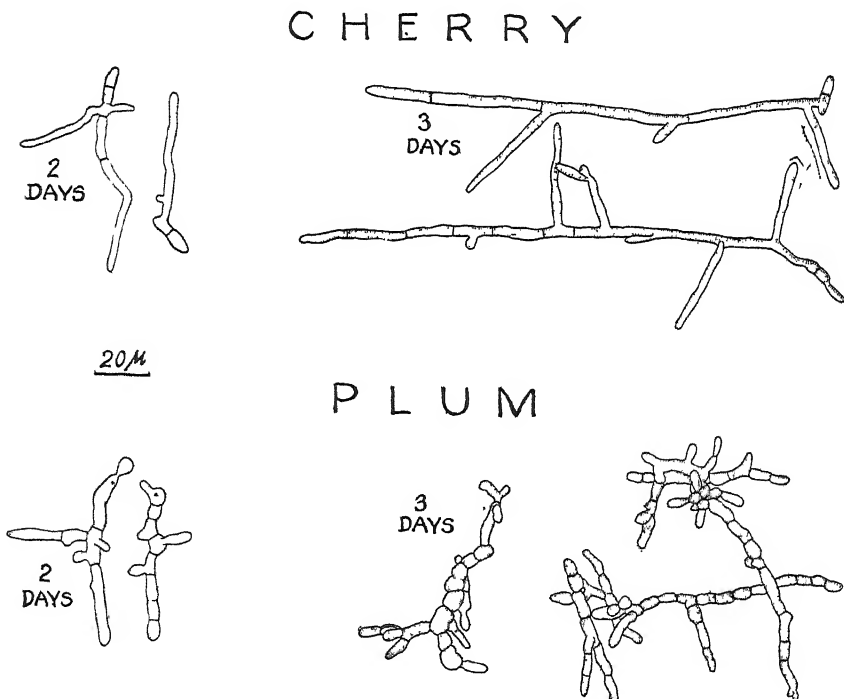


FIG. 7. Young thalli of *Cladosporium* from cherry (*P. cerasus*) and plum (*P. americana*) after stated periods of growth on plates of 2 per cent agar in water at 24° C. Traced from photomicrographs.

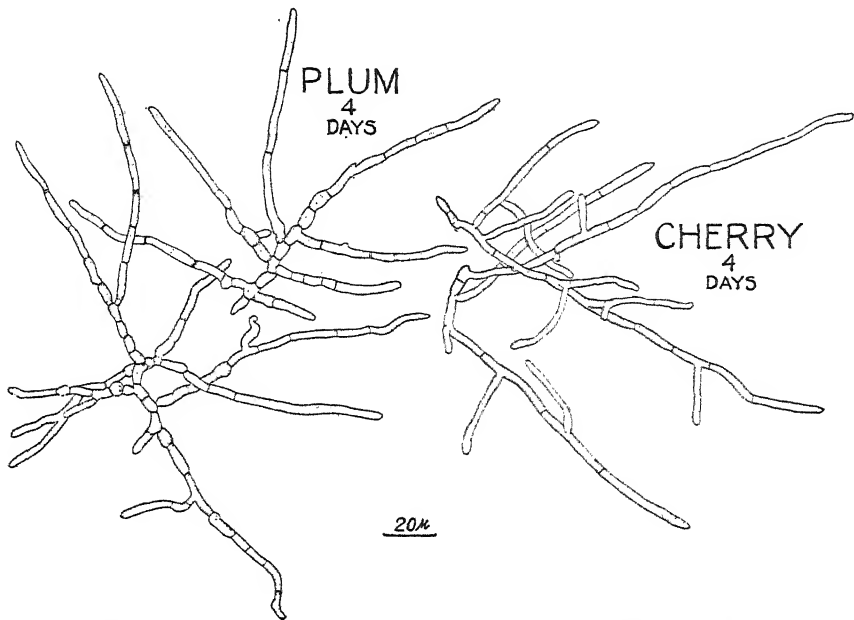


FIG. 8. Young thalli of *Cladosporium* from plum (*P. americana*) and cherry (*P. cerasus*) grown on plates of 2 per cent agar in water at 24° C. Traced from photomicrographs.

DISCUSSION

The results herein reported furnish further evidence of the general similarity of the *Cladosporium* diseases studied. In each case the fruit, leaves, and twigs of the host plant are attacked, the general relations of host and parasite appear to be similar, and the lesions produced differ only in detail. Certain differences are reported, however, which seem to segregate these diseases into two groups: (1) scab of *A. persica* and *P. americana* (and probably other hosts to which these studies were not extended), and (2) scab of *P. cerasus*. From the ease with which *P. armeniaca* was infected with strains of the fungus from *A. persica* and *P. americana*, it seems most likely that the apricot disease falls in the group with peach and plum scab.

In these studies no significant differences have been observed in the *Cladosporium* from *A. persica* and *P. americana* or in the symptoms which they induced.

The cherry *Cladosporium* differed from that of peach and plum in several respects. In the cross-inoculation experiments it induced the disease only on *P. cerasus*, giving negative results (save for some penetration of the

cuticle and slight subcuticular development) on *A. persica*, *P. americana*, and *P. armeniaca*. In contrast, the strains from *A. persica* and *P. americana* readily infected *A. persica*, *P. americana*, and *P. armeniaca*, but failed to induce typical infection or sporulation on *P. cerasus*. Under the conditions of these tests the temperature range for germination and growth of the fungus from *P. cerasus* seemed to be distinctly lower than that for the *Cladosporium* from *A. persica*. The cherry fungus also differed from the others in the form of the germ tubes and of the young thalli developed in cultures. While these data seem to show clearly that these fungi fall into two groups in their pathological and physiological relationships, they do not constitute a satisfactory basis for judgment as to the genetic and taxonomic relationships of these parasites. The cherry *Cladosporium* conforms with Aderhold's (1, p. 542) description of the imperfect stage of *Venturia cerasi* Aderh., with which it is probably identical. Since ascigerous stages have been found for certain closely related fungi, as the apple, pear, and cherry scab parasites, it seems logical to suspect that the *Cladosporiums* of peach, plum, and other stone fruits may have similar genetic connections. Until further information relating to these possibilities is available, it would seem that the cherry *Cladosporium* with which we worked should be referred to *Venturia cerasi* Aderh. (synonym: *Cladosporium cerasi* (Rbh.) Sacc.), and the strains from *A. persica* and *P. americana* to *Cladosporium carpophilum* Thüm.

SUMMARY AND CONCLUSIONS

1. The symptoms induced by species of *Cladosporium* on *P. americana*, *P. armeniaca*, and *P. cerasus* are described.
2. The *Cladosporiums* studied infect the leaves of their hosts by direct penetration of the cuticle by infection hyphae from closely appressed and strongly adherent germ tubes.
3. In cross-inoculation experiments, strains of *Cladosporium* from *A. persica* and *P. americana* infected *A. persica*, *P. americana*, and *P. armeniaca*, but failed to infect *P. cerasus* or *P. domestica*. The *Cladosporium* from *P. cerasus* infected its own host, but failed to induce typical infection or sporulation on *A. persica*, *P. americana*, or *P. armeniaca*. Infection hyphae from germ tubes of the *Cladosporium* from *P. americana* readily penetrated the cuticle of leaves of *P. cerasus*, but failed to develop sufficiently to fructify or to induce macroscopic lesions. Similar cases of penetration were observed in other cross-inoculation trials where negative results were obtained.
4. The *Cladosporium* from *P. cerasus* appears to have a distinctly lower thermal range for germination of conidia and for vegetative development than has the *Cladosporium* from *P. americana*.

5. The *Cladosporium* from *P. cerasus* differed from the strains from *A. persica* and *P. americana* in the form of germ tubes and of young thalli developed in cultures.

6. Measurements of conidia of strains of *Cladosporium* from *A. persica* and *P. americana* agreed very closely. The conidia of the *Cladosporium* from *P. cerasus* were slightly shorter, but the significance of this difference is doubtful.

7. Pathologically and physiologically, the *Cladosporiums* studied appear to fall into two groups: (1) those from *A. persica* and *P. americana*, and (2) that from *P. cerasus*. Their genetic and taxonomic relationships are likely to be somewhat doubtful until more is known of their possible ascigerous stages. Meanwhile, the fungi from *A. persica* and *P. americana* are tentatively regarded as identical and are referred to *Cladosporium carpophilum* Thüm.; and the cherry fungus is tentatively referred to *Venturia cerasi* Aderh. (synonym: *Cladosporium cerasi* (Rbh.) Sacc.).

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A STUDY OF PATHOGENIC AND NON-PATHOGENIC STRAINS OF PSEUDOMONAS TUMEFACIENS SM. & TOWN.

M. K. PATEL¹

INTRODUCTION

The longevity, distribution, and overwintering of *Pseudomonas tumefaciens* Sm. and Town. in soils of different types is a matter of great economic importance, although there is comparatively little known about any of the activities of the organism in the soil. This lack of knowledge is probably due to the difficulties involved in readily recovering the crown gall organism from soils. The method of detecting a particular pathogene in the soil by gross inoculation of soil suspensions into susceptible hosts is open to serious criticism. The development of a method by which *Ps. tumefaciens* may be readily isolated from soils has made it possible to accumulate much new information about the activity of the crown gall organism in the soil.

Riker (10), Reddick and Stewart (9), and Muncie (6) have reported work on one or another phase of this problem under natural and artificial conditions in different types of soils, but as yet no study has been carried out on the distribution both of virulent and non-virulent strains of *Pseudomonas tumefaciens* in soils which have been growing different crops, as, for example, virgin soils, nursery soil, pasture, and corn-land. The results of such a study, together with data on the overwintering of the pathogene in sterilized and unsterilized soils of various types, are reported in this paper.

Before discussing the results obtained, the literature bearing directly on the subject will be briefly summarized.

LITERATURE REVIEW

Fulton (2), working with the citrus canker organism, *Pseudomonas citri* Hasse, found that the number of pathogenic organisms in artificially infested soil decreased with time. The pathogene had disappeared after two weeks had elapsed. In order to prove the presence or absence of the organism in question, he inoculated mature leaves of grapefruit with soil suspensions.

¹ The writer wishes to express his gratitude to Drs. I. E. Melhus and J. H. Muncie for valuable criticisms and suggestions made from time to time during the progress of this work. These studies have been carried out in connection with the crown gall project in which the Crop Protection Institute of the National Research Council; United States Department of Agriculture, Office of Mycology and Disease Survey; Iowa State College; and University of Wisconsin are cooperating.

In the case of potato black-leg, caused by *Bacillus atrosepticus* van Hall, Rosenbaum and Ramsey (11) were unable to reisolate the organism from the soil of their wintering-over test plots. Kotila and Coons (4), while able to prove the presence of *B. atrosepticus* in the soil immediately surrounding diseased plants by inoculating potato slices directly with soil particles, found that the organism either quickly disappeared from the soil or lost its pathogenicity.

Goldsworthy (3), working with the longevity of the cauliflower spot disease organism, *Pseudomonas maculicolum* McCulloch, in the soil, attempted to recognize the presence of the pathogene on poured plates by means of agglutination phenomena. He concluded that typical colonies, which he isolated from such plates, that gave the same agglutination titre as the pathogene in question were identical species. This conclusion is open to the criticism that no inoculation experiments are reported with these organisms and, as Durham (1), Stevens (13), and Wilson (14) have shown, that different varieties and even species may agglutinate at the same titre. More conclusive results would doubtless be reached by a combination of agglutination and inoculation.

In 1922, Riker (10) showed that the crown gall organism retained its viability for a year in sterilized soil. Reddick and Stewart (9), also using sterilized soil, were able to isolate *Pseudomonas tumefaciens* from clay after 43 days, from loam and quartz sand after 186 days. They failed, however, to obtain the organism after 298 days. The data from similar experiments on unsterilized soils were unfortunately lost. In addition to these data on longevity, they showed, that, when free from competition, *Ps. tumefaciens* could withstand low temperatures and repeated changes of temperature at or near the freezing point in the soil and that it might move considerable distances with the currents of soil water. Their criterion of viability was successful inoculation of tomato plants by means of soil suspensions.

While all these workers studied the longevity of the crown gall organism in sterile soil, Muncie (5), in one experiment, recorded the presence of the viable pathogene in unsterilized soil after 102 days, and later (6), in a second trial, obtained successful inoculations with a suspension from an unsterilized loam which had been infested 154 days previously. More recently the writer (8) successfully isolated *Ps. tumefaciens* in a virulent state from unsterilized soils 10 months after their infestation. Since this report these studies have been carried further, and the results are presented in this paper.

MATERIALS AND METHODS

Soils

The experiments reported in this paper were carried out on three types of soil in the laboratory: clay, loam, and sand. The clay was a sub-soil clay

collected from a building excavation at Ames, Iowa. The loam was a greenhouse soil, high in organic matter. The sand was quartz sand.

In addition to the laboratory trials, soils in two different fields of the silt loam type were artificially infested. In the first field the infestation was produced by means of pure culture, and in the second field by infected plant materials.

Each of the three soils used in the laboratory was divided into two parts and placed in flasks of 300 cc. capacity. One portion was sterilized in the autoclave at 20 pounds for 6 hours. Sterilization was tested by means of poured plates using potato dextrose agar as a medium. No colonies were found on these plates after 6 days. Both portions were then infested by introducing into the flasks enough of a 72-hour-old broth culture of *Ps. tumefaciens* to wet the samples thoroughly. The flasks were stored in an ice-box at 12-14° C. The moisture content was maintained by the addition of sterile distilled water to the flasks as need arose.

For the overwintering studies, similar soils, that is, clay, loam, and sand, sterilized and unsterilized, were artificially infested with a 72-hour-old broth culture of *Ps. tumefaciens* after they had been tubed. Fifteen tubes were filled to a depth of 3 inches with each kind of soil, infested, and buried at a depth of 6 inches on October 5, 1926. These tubes remained in the ground all winter. On March 10, 1927, they were dug up, and isolations were made from their contents.

Besides the soil cultures that were buried in the ground, 10 tubes of a sterilized greenhouse loam and 10 tubes of unsterilized greenhouse loam were artificially infested with a 72-hour-old broth culture of the crown gall organism and placed in two pots on the surface of the ground outside the greenhouse. The tubes were covered with soil in the pot, but the pots were left uncovered. These pots were placed outdoors on December 10, 1926. Tubes were removed at intervals up to March 10, 1927, when the trial was finished. The tubes were about half filled with soil.

Finally two soil samples furnished by Muncie (6) were added to the series of soils mentioned above. These samples consisted of clay and greenhouse loam, sterilized and unsterilized, infested on January 13, 1926. These samples were carried in the laboratory in the same manner as the flasks, and isolations were made at irregular intervals.

Isolations

Since the object of the isolations was to secure a specific organism, *Ps. tumefaciens*, and not, as with the usual soil bacteriologic technic, the total number of organisms present, a special method was developed which took advantage of certain peculiarities of the nutritive requirements of this or-

ganism. With the larger samples, approximately 20 grams of the soil sample were dropped into a bottle containing 100 cc. of sterile distilled water and shaken for 2-5 minutes. The bottle was then allowed to stand for from 3 to 8 hours with occasional shaking. After a very vigorous final shaking the bottle was rested for 10 minutes to allow the heavy particles of soil to settle out. From the supernatant, but still cloudy, liquid, dilutions of 1-1000, 1-10,000 and 1-100,000 were made, using sterile pipettes and sterile distilled water, and the usual other precautions to maintain aseptic conditions during transfer. Ordinarily three plates were poured from each dilution, using crystal violet bile agar as devised and already described by the author (7), as follows: 1 cc. of the dilution to be poured was added to a flask containing 25 cc. of crystal violet bile agar, and, after thorough mixing, the contents were poured into three sterile petri dishes using approximately one-third of the mixture for each dish. After the agar had solidified the plates were inverted and incubated at room temperature (*circa* 25° C.). After 48 hours the plates were examined daily with a hand lens for 10 days or more. Usually the colonies of *Ps. tumefaciens* came up in 48-72 hours in this medium.

As a check on the presence of the crown gall organism in the dilutions, three healthy young tomato plants were inoculated with each dilution of soil by dipping a sterile needle into the soil suspension and then scratching the upper portion of the stem of the plants in from 5 to 10 places.

Later, when colonies resembling *Ps. tumefaciens* appeared on the plates, they were marked and inoculated into three tomato plants by puncturing the tender portion of the top. The plants were cared for in the greenhouse under conditions suitable for continued, rapid, succulent growth. Trial plants were examined for galls at intervals during three to six weeks and were then discarded. Care was taken to prevent accidental infection from soil splashing or by other means, by separating the plants from one another by 3 inches in all directions.

Crystal violet bile agar was used as the medium for the isolations because, as reported in a previous paper (7), this medium eliminates gram positive bacteria and most of the coccus forms and checks the growth of the molds. The use of potato dextrose agar which was used in the earlier isolation studies was discontinued since *Ps. tumefaciens* grows more slowly on this medium than on the crystal violet bile agar, and the various contaminants—spreaders, molds, etc.—grew on it so well that the plates soon became overrun with these forms, making close examination impossible.

LONGEVITY OF *PSEUDOMONAS TUMEFACIENS* IN SOILS IN THE LABORATORY

The longevity of *Pseudomonas tumefaciens* in the laboratory was tested in sterilized and non-sterilized clay, loam, and quartz sand. The results of these experiments are presented in table 1.

TABLE 1.—*Longevity of Pseudomonas tumefaciens Sm. and Town. in sterilized and non-sterilized soils at room temperature in the laboratory as determined by isolation and inoculation of the organisms into tomato plants*

Date of infection	Date of isolation	No. days organisms in soil	No. plants inoculated from each sample	No. plants infected with colonies from						No. controls infected
				Sterilized			Unsterilized			
				clay infested	loam infested	sand infested	clay infested	loam infested	sand infested	
June 3, 1926	Aug. 31, 1926	90	3	3	3	3	3	3	3	0
do	Oct. 2, 1926	122	3	3	3	3	3	3	3	0
do	Nov. 10, 1926	160	3	3	3	3	0	3	3	0
do	Mar. 1, 1927	271	3	3	3	3	2	3	2	0
do	Mar. 25, 1927	295	3	3	3	3	0	2	2	0
do	April 10, 1927	311	3	3	3	3	0	3	3	0
do	May 17, 1927	349	6	6	6	6	1	3	5	0
do	June 8, 1927	371	6	6	6	6	0	1	3	0
do	July 26, 1927	420	6	6	6	6	1	1	2	0
do	Aug. 31, 1926	231	3	3	0	...	0
^a Jan. 13, 1926	Oct. 2, 1926	263	3	0	0	...	0
do	Nov. 2, 1926	294	3	0	3	...	0
do	Nov. 10, 1926	302	3	0	3	...	0
do	Mar. 14, 1927	424	5	0	1	...	0
do	May 17, 1927	491	6	0	0	...	0

^a These soil samples were kindly supplied by Dr. J. H. Muncie and were used in his studies (6, p. 83, Table V).

From these data it will be seen that, under the conditions of these trials, *Ps. tumefaciens* was still alive and active for many days after the soil was infested. In the series infested on June 3, 1926, the organism was still viable after 420 days in all three types of soil which had been sterilized before infestation. In the case of the soils which were not sterilized the data show that the pathogene decreased in numbers in the clay much more rapidly than in the loam or sand, although one successful infection was obtained from six inoculations made after 420 days. In the case of the loam and sand, inoculations made after 420 days were also successful, although there is some evidence of attenuation in that the number of infections is reduced as the time between infestation and inoculation increases.

The two soil cultures infested and reported on by Muncie (6) were continued, and after 231 days the organism was found in the sterilized clay. Unfortunately this soil sample became contaminated by a mold, and no inoculations produced infection thereafter. With the other culture of unsterilized loam, tomatoes became infected when inoculated after 424 days, although the gall produced in the last trial was very small. An isolation from this gall yielded a pure culture of the pathogene, which in turn produced typical crown gall on inoculated tomato plants.

These experiments show that *Ps. tumefaciens* may live for a year at least in both sterile and unsterilized soils in the laboratory. It also seems clear that it will live longer in sterile soil, where it is not in competition with other organisms, and that it may live longer in sandy soils than in clays. That the pathogene becomes attenuated in the unsterilized clay, loam, and sand is shown by the gradual reduction in the number of successful inoculations from these soils. No data were obtained by counting the number of organisms in the soil, since this method was regarded as too unreliable due to the difficulty of recognizing the crown gall organism with sufficient accuracy.

When one considers the fact that each different lot of soil was infested with an approximately equal number of crown gall bacteria in an equal volume of medium, it would seem that competition existing between the micro-organisms in the soil has a greater influence upon the longevity of *Ps. tumefaciens* than the apparent nutritive content of the soil medium. This may account for the gradual decrease in the number of infections obtained on the tomatoes inoculated from suspensions of infested clay as compared with sand, since clay normally harbors greater numbers of micro-organisms.

OVERWINTERING OF PSEUDOMONAS TUMEFACIENS IN THE OPEN

It has been shown above that the crown gall organism can live in the soil under laboratory conditions for more than a year. However, these

conditions did not subject the organism to so severe a test as would occur in the field. Therefore samples of sterilized and unsterilized soil were infested with *Ps. tumefaciens* and exposed to outside conditions as already described. These samples were either free in the field or confined in glass containers. Isolations were made at various times from those soil samples kept on the surface of the soil, and the organisms isolated were inoculated into healthy tomato plants. Inoculations were also made directly from the soil suspensions, and infection was obtained on tomatoes inoculated from each sample. Three control plants were held for each inoculation. The results of these trials are given in table 2.

From these data it is clear that *Ps. tumefaciens* can live over winter in the soil, since all five trials with both soils yielded the pathogene after 5, 18, 26, 76, and 90 days. The temperature during this period ranged from -23° C. on December 15, the lowest temperature noted during this time, to 15° C. on January 2, 1927.

The temperature records from October 5, 1926, to March 10, 1927, were obtained from the Weather Bureau station of the United States Department of Agriculture at Ames, Iowa.

Except for three days, the minimum daily temperatures in the month of October, 1926, were above freezing. These ranged from 15° C. to -5° C., the maximum daily temperatures for the same period ranging from 25° C.

TABLE 2.—*The overwintering of Pseudomonas tumefaciens Sm. and Town., out of doors, as determined by inoculations with organisms isolated from unsterilized and sterilized soils infested Dec. 10, 1926*

Kind of soil	No. of samples	Date of isolation	No. days outdoors	No. tomatoes inoculated	No. tomatoes infected	No. controls infected
Unsterilized						
greenhouse loam	1	Dec. 10, 1926	0	3	3	0
do	1	Dec. 15, 1926	5	3	3	0
do	1	Dec. 28, 1926	18	3	3	0
do	1	Jan. 5, 1927	26	3	3	0
do	1	Feb. 24, 1927	76	3	3	0
do	5	Mar. 10, 1927	90	6	6	0
Sterilized						
greenhouse loam	1	Dec. 10, 1926	0	3	3	0
do	1	Dec. 15, 1926	5	3	3	0
do	1	Dec. 28, 1926	18	3	3	0
do	1	Jan. 5, 1927	26	3	3	0
do	1	Feb. 25, 1927	76	3	3	0
do	5	Mar. 10, 1927	90	3	3	0

to 4° C. In November, the minimum temperatures were at or below freezing, except for 5 days. These ranged from 9° C. to -12° C. The maximum temperature for the same period ranged from 18° C. to -5° C., being below zero for 5 days at random.

The minimum daily temperatures in the month of December were below freezing, ranging from 0° C. to -22° C. The maximum temperatures for the same period were at, or slightly below, freezing except for 14 days in which they ranged from 13° C. to -13° C. The minimum temperatures in January, 1927, were below freezing, ranging from 2° C. to -23° C., except for 2 days when it registered 2° C. and 1° C. The maximum temperatures during this month were above freezing except for 12 days. These ranged from 10° C. to -13° C. During this month the minimum daily temperature reached the lowest point for the entire period of the experiment. The maximum temperatures in February were above freezing—14° C. to -7° C.—except for 4 days. The minimum temperatures for the same period were below freezing except for 3 days, ranging from -18° C. to 2° C. This month marked the rise in temperature which continued up to March. During March the maximum temperatures were above freezing except for 1 day. The minimum temperatures for the first 10 days of the month ranged from -10° C. to 3° C.

There was, during the course of the experiment, no period of more than 6 days in which the maximum daily temperatures remained continuously

TABLE 3.—Overwintering of *Pseudomonas tumefaciens* Sm. and Town., as determined by inoculations with organisms isolated from sterilized and non-sterilized soils buried 6 inches in the soil out of doors on Oct. 5, 1936

Kind of soil	No. of samples	No. days outdoors	No. tomatoes inoculated	No. tomatoes infected from soil suspensions	No. tomatoes infected from plated colonies	No. controls infected
Sterilized sand	13	156	17	13	4	0
Sterilized clay	12	156	15	9	5	0
Sterilized loam	12	156	17	5	11	0
Non-sterilized sand	15	156	22	10	8	0
Non-sterilized clay	12	156	17	11	6	0
Non-sterilized loam	12	156	18	9	9	0

below the freezing point. During the months of December and January the temperature reached the lowest maximum and minimum marks. The fluctuations during these months were numerous and abrupt.

In the case of the buried samples, isolations were made 156 days after they were placed underground. The results of these isolations and inoculations into tomato plants are shown in table 3. It will be noted that in all cases the pathogene was reisolated from the soils and successfully reinoculated into tomato plants. In a few cases the first galls produced from the reisolation cultures were very small. In such instances the organism was reisolated from the gall and used in inoculating other tomato plants. Normal-sized galls resulted in all these inoculations. Recovery of the pathogene was somewhat more difficult from the tubes of sterilized soil which were plugged with cotton and buried outside, because the cultures were contaminated when the plugs became soaked with soil water.

Further to substantiate the possibility of the overwintering of the crown gall pathogene, soil in the field was infested with the bacterium both from pure cultures in broth and from finely chopped tomato galls ploughed into the soil on October 5, 1926. The reisolations and inoculations from the soils of these field plots were made on March 10, 1927, after an elapse of 156 days. In this instance successful inoculations from the soil suspensions were made only in the case in which pure cultures had been used for the source of infestation. However, virulent colonies of *Ps. tumefaciens* were isolated from soil subjected to both types of infestation. Smith, Brown, and Townsend (12) reported the successful renewal of growth of *Ps. tumefaciens* in pure culture after it was subjected to a temperature of from 0° C. to -14° C. for two weeks.

In another experiment, 4 castor bean plants outside the greenhouse were inoculated with *Ps. tumefaciens* at internodes some time during the early fall. The galls had grown to a size of about 2 cm. before winter set in and the plants were killed. From time to time during the winter months isolations were made from the galls on the dead host and in no case was the pathogene recovered. It is possible that the crown gall pathogene does not live over winter on a dead host under Iowa conditions.

From these experiments it is clear that *Ps. tumefaciens* can overwinter readily in the soil in the absence of its host.

DISTRIBUTION OF PSEUDOMONAS TUMEFACIENS IN SOILS BEARING VARIOUS CROPS

In addition to the studies on longevity and overwintering of *Ps. tumefaciens* in the soil, a survey was made of soils from 12 nurseries representing 9 states to determine, if possible, the distribution of the crown gall organism in these soils, and the relation of its presence to the crop being grown on the soil.

The samples of soil from 12 different nurseries in 9 states were collected by Dr. Muncie and Messrs. Layton, Shippy, and Johnston, while on crown gall survey in the field. They took care that the samples were kept separate. As soon as they reached the laboratory, the soils were placed in pots in the greenhouse and kept moist. Isolations were made in the same manner as in the longevity studies, and inoculations were made from soil suspensions and from colonies suspected of being *Ps. tumefaciens*.

The description of the soils used and the results of the isolations and inoculations are tabulated in table 4.

TABLE 4.—*Distribution of Pseudomonas tumefaciens in various field soils*

Culture no.	No. of samples	No. of trials	Present crop	Past crop	Crowngall observations	Presence of organisms resembling the pathogene in plates ^a	Infection on tomatoes from colonies and soil suspension ^b
A-1	1	2	Farm crops	Farm crops	Absent	—	—
A-2	1	2	Pear	Pear	Abundant	+	—
B-1	1	—	Apple	Farm crops	Slight	0	0
B-2	1	1	do	do	do	+	—
B-3	1	1	Evergreen	Evergreen	Absent	—	—
B-4	1	1	Rose	Rose	Badly galled	+	—
C-1	1	5	Evergreen	Evergreen	Absent	—	—
C-2	1	3	Apple	Ornamentals	Slight	+	—
C-3	1	3	Cherry	do	do	+2	—
C-4	1	3	Apple	do	do	+3	+2
C-5	1	3	do	do	13 per cent	+3	+2
D-1	1	1	do	do	Badly galled	—	—
D-2	1	1	do	do	Slight	+	—
D-3	1	1	Evergreen	Evergreen	Absent	+	—
D-4	1	1	Rose	Rose	Badly galled	+	—
E-1	1	2	Poplar	Poplar	Absent	—	—
E-2	1	1	Peach	Peach	Slight	—	—
F-1	1	1	Apple	Old orchard	do	+	—
F-2	1	1	do	Nursery stock	do	—	—
F-3	1	1	do	do	do	—	—
F-4	1	1	do	Pasture	Absent	—	—
F-5	1	1	do	Apple	Badly galled	+	—
F-6	1	1	Peach	Nursery stock	Absent	+	—
F-7	1	1	Apple	do	do	—	—
F-8	1	1	do	Pasture	Some	+	—
G-1	1	1	Peach	Nursery stock	Badly galled	—	—
G-2	1	1	do	do	do	—	—
G-3	1	2	do	do	Clean	—	—
G-4	1	3	do	do	10 per cent	+2	+2
H-1	1	1	Apple	do	Moderate	—	—
H-2	1	1	do	do	do	—	—
H-3	1	1	do	do	do	+	+
H-4	1	1	do	do	do	+	+
H-5	1	1	do	do	do	+	—
H-6	1	1	do	do	do	+2	+2
H-7	1	1	do	do	do	+	—

TABLE 4.—(Continued)

Culture no.	No. of samples	No. of trials	Present crop	Past crop	Crown gall observations	Presence of organism resembling the pathogene in plates ^a	Infection on tomatoes from colonies and soil suspension ^b
H-8	1	1	Apple	Nursery stock	Moderate	+	-
H-9	1	1	do	do	do	+	-
H-10	1	1	do	do	do	+	+
H-11	1	1	do	do	do	+	-
H-12	1	1	do	do	do	+	-
H-13	1	1	do	do	do	+	-
I-1	1	1	do	do	Slight	-	-
I-2	1	1	do	do	do	-	-
I-3	1	1	do	do	Clean	-	-
I-4	1	1	do	do	do	-	-
I-5	1	1	Peach	Ornamentals	Slight	-	-
J-1	1	1	Corn	Apple	Badly galled	+	-
J-2	1	1	Apple	do	Slight	+	-
K	9	9	Peach	Peach	Clean	+2	-
L	6	6	Apple	Apple	Absent	+2	-
M-1	1	1	Peach	No nursery stock	do	+	-
M-2	1	1	do	do	do	+	-
N-1	13	13	Apple	Apple	Clean	+3	-
N-2	4	4	do	do	Slight	+4	-
O-1	1	1	do	do	do	-	-
O-2	1	1	Evergreen	Evergreen	Absent	-	-
P-1	3	3	Corn	Corn	do	-	-
P-2	3	3	None	None	do	-	-
P-3	3	3	do	do	do	-	-
P-4	3	3	Apple	Apple	do	+1	-

^a—denotes the absence and + denotes the presence of organisms resembling *Ps. tumefaciens*. A number accompanying the sign denotes the number of trials made.

^b Infections from colonies and soil suspensions were identical. No infection is indicated by - and infection by +. A number accompanying the sign denotes the number of trials made; 0 denotes no inoculation.

With the exception of samples F8 and K, which were of a sandy nature, the soil type in the nurseries was a heavy loam. In all cases except K, L, and M, the soil had been fertilized by turning under a green manure crop not more than three years before the samples were taken.

These data show that the organisms resembling *Ps. tumefaciens* are not generally found in soils from which crops susceptible to this pathogene are absent. From only 1 of 14 samples was an organism of this type isolated. Tomato plants inoculated with this organism did not become infected.

On the other hand, organisms resembling *Ps. tumefaciens* were isolated 41 times from 96 soil samples; and of the 41 cultures, 7 proved pathogenic for tomato upon inoculation.

The data also indicate that the pathogenic form of *Ps. tumefaciens* is localized in the field, because infection was obtained after inoculation with soil suspensions only in cases in which the samples had been in close proximity to true galls. Soil suspensions from samples taken at a distance from the crown-galled trees did not infect tomatoes upon inoculation.

In order to eliminate the possibility of the occurrence of organisms resembling *Pseudomonas tumefaciens* as contaminants in air, water, or common disinfectants used in the experiments, four trials were made to detect the presence of such organisms in these materials. Potato dextrose plates were poured and exposed to the air from 5 to 20 minutes outdoors and in the laboratory. The tap water and disinfectants were treated as soil suspensions. Organisms resembling *Ps. tumefaciens* were found in no case.

SUMMARY

Pure virulent cultures of *Ps. tumefaciens* were recovered from infested sterilized clay, loam, and quartz sand after 420 days. The infested unsterilized samples of clay, loam, and quartz sand also yielded the pathogene after 420 days, but the number of pathogenic colonies in these samples decreased in the order of clay, loam, and sand. Infection was produced from another sample of infested unsterilized clay after 424 days.

In the overwintering studies *Ps. tumefaciens* was recovered from artificially infested soils after being subjected for 90 days to out-of-door temperatures ranging from -23° C. to 15° C. From infested samples buried at a depth of about 6 inches in the soil the organism was recovered after 156 days of winter temperatures. In the field in soils infested with pure cultures and in others infested with diseased tissue, the organism was recovered in both cases after passing the winter in the soil in the absence of the living host.

Organisms resembling *Ps. tumefaciens* were recovered in 41 cases from 96 nursery soil samples; and of these, 7 proved pathogenic for tomato upon inoculation. In each case the pathogene was recovered from soils upon which hosts susceptible to *Ps. tumefaciens* were growing. Suspensions of 14 samples of soil on which non-susceptible crops were grown for a number of years did not infect tomatoes. No colonies resembling *Ps. tumefaciens* were obtained from the air, tap water, or common disinfectants.

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PHYSIOLOGIC SPECIALIZATION IN PUCCINIA SORGHI¹

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INTRODUCTION

In recent years considerable work has been done in developing disease-resistant lines of corn. But relatively little attention has been paid to corn rust, caused by *Puccinia sorghi* Schw., because it has caused so little injury that it is generally considered of only minor economic importance. However, Stakman and Christensen (9) pointed out that the rust might be very destructive to certain selfed lines of corn and should be taken into consideration in any program of corn breeding. For this reason the writers obtained data on the relative resistance to rust of a large number of selfed lines of different varieties of corn and made attempts to ascertain whether there was any correlation between the reaction of the lines to rust and smut. In addition, there was some field evidence that there might be physiologic forms of *P. sorghi*, and a study therefore was made to find out whether there actually were such forms.

FIELD STUDIES ON THE RELATIVE RESISTANCE OF SELFED LINES

In 1923 there were striking differences in the rust reaction of selfed lines of Squaw Flint grown in the plant breeding nursery at University Farm, St. Paul, Minnesota. In 1924 rust inoculum was apparently not so abundant as in the previous year, although one selfed strain of Northwestern Dent was rather heavily infected. In 1925 a few selfed strains of Squaw Flint again were heavily infected. The leaves, husks, necks, and tassels of these strains were so severely affected that the plants died prematurely. There was considerable variation in the reaction of other selfed lines of Squaw Flint and of certain inbred lines of other varieties. (See figures 1 and 2.)

As rust seldom seemed to be destructive to ordinary varieties of corn grown in the field, the severity of attack on some of the selfed lines sug-

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The writers are indebted to W. Butler, J. H. Craigie, and H. A. Rodenhiser for collections of rusts.

The work was done as one phase of a project on the development of disease-resistant varieties of farm crops, carried on cooperatively by the Sections of Plant Genetics and Plant Pathology. The field observations were made in the regular corn breeding plots of the Section of Plant Genetics.

gested the necessity of taking into consideration susceptibility to rust in selecting the better lines.

Conclusions regarding varietal resistance of corn to diseases are not generally considered very reliable because corn usually is very heterozygous. But Weber (12), in 1921, noted differences in the susceptibility of the several species of corn to rust. *Zea everta* (pop-corn) was the most resistant, while *Z. saccharata* (sweet corn) seemed to be the most susceptible. Weber also tested six varieties of dent corn but found only slight differences in their reaction to rust. Furthermore, field observations made over a period of years in Minnesota indicated that there were marked differences in varietal susceptibility to rust, both of field corn and sweet corn. Of 22 varieties of sweet corn tested, Golden Bantam was by far the most susceptible, while Crosby and Country Gentleman were quite resistant. Squaw Flint was the most susceptible variety of field corn under observation. In general, most of the varieties of field corn grown in Minnesota appear to be sufficiently resistant to remain uninjured in the field.

In 1924, Mains et al. (8) and Holbert et al. (4) called attention to striking differences in the susceptibility of different selfed lines of corn. Stakman and Christensen (9), in 1926, also pointed out that there were

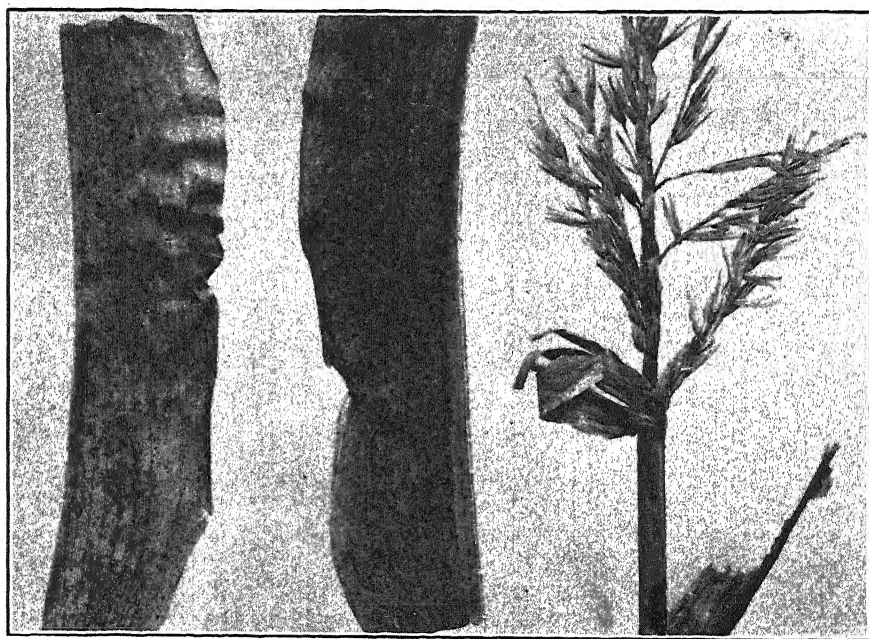


FIG. 1. Minn. No. 13, culture 86, selfed one year. Severely attacked by corn rust.
A. Lower side of leaf. B. Upper side of leaf. C. Tassel infection.

great differences in the susceptibility of different selfed lines. It seemed desirable, therefore, to obtain as much information as possible regarding the relative susceptibility of the selfed lines which were grown in the plant breeding plots at University Farm.

The lines of corn studied during the summer of 1925 had been selfed from 2 to 7 years, most of them from 4 to 5. About 40 plants of each line were grown. They were planted 1 foot apart in rows $3\frac{1}{2}$ feet apart. Observations were made on the degree of natural infection of rust and smut on 171 of these lines. The data are summarized in table 1.

TABLE 1.—*The reaction of 171 selfed lines of corn to rust and smut in the plant breeding plots at University Farm, St. Paul, Minnesota, 1925*

Variety	No. lines tested	Years selfed	Infection classes and number of lines in each class					
			Rust			Smut		
			Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible
Minn. No. 13	17	5 ^a	6	9	2	7	8	2
Longfellow ...	18	2 to 6	5	12	1	5	8	5
Northwestern								
Dent	18	5 to 7	10	8	0	9	7	2
Squaw	46	2 to 7	3	23	20	13	17	16
Rustler	18	5 to 7	13	5	0	8	8	2
King Phillip	39	3 to 6	11	28	0	8	8	23
Minn. No. 23	15	3	12	3	0	0	9	6
Total	171		60	88	23	50	65	56

^a Except two lines which were selfed 1 and 2 years respectively.

There were marked differences in resistance and susceptibility, both to rust and smut. It is well known that there is great variation in smut reaction of selfed lines obtained from a commercial variety, and the need of selecting for smut resistance is well recognized (2, 3, 5, 6).

Reaction to rust within inbred lines has not been studied so extensively as that to smut. From observations made in the plant breeding nursery since 1923 it appears that susceptible lines of Squaw are relatively numerous but that highly susceptible inbred lines of other varieties are rather uncommon. Susceptibility or resistance to smut appeared to be entirely independent of the reaction to rust, and many lines which appeared resistant to both diseases were found (table 2).

There were many different types of rust reaction. On some of the lines there were relatively few, but very large, pustules; on other lines there were numerous small pustules; on others there were large pustules sur-

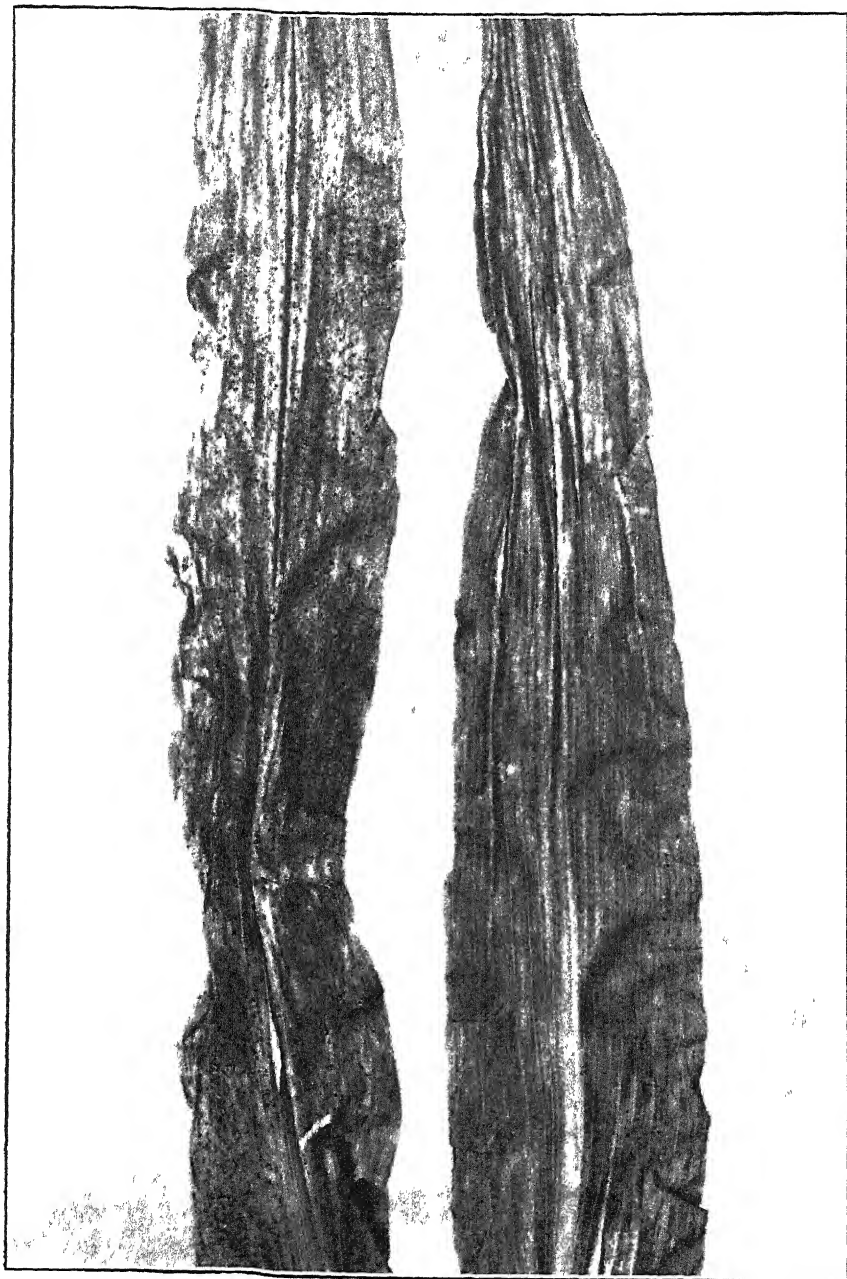


FIG. 2. Two selfed lines of corn, showing difference in susceptibility to rust under field conditions, 1925. A (Culture 1078) 7 years selfed, susceptible
B (Culture 1054) 3 years selfed, resistant.

TABLE 2.—*Relation between degree of rust infection and degree of smut infection in the 171 selfed lines of corn listed in table 1*

		Reaction to rust			Total
		Resistant	Intermediate	Susceptible	
Reaction to smut	Resistant	18	23	9	50
	Intermediate	26	30	9	65
	Susceptible	16	35	5	56
Total		60	88	23	171

rounded by distinct necrotic areas; and on still other lines there were both large and small pustules, either with or without chlorosis or necrosis. This suggested the presence of two or more physiologic forms of *P. sorghi*.

PHYSIOLOGIC SPECIALIZATION WITHIN PUCCINIA SORGHI

In 1926 Stakman and Christensen (9) published a preliminary note on physiologic specialization in *Puccinia sorghi* and *Ustilago zeae*; and Mains (7) also showed that there are forms of *P. sorghi*. The experiments recorded in the present paper are a continuation of work begun in 1924, when two forms were isolated, one of which infected teosinte heavily, while the other infected it weakly.

Materials and Methods

Numerous collections of *P. sorghi* were obtained from the Mississippi Valley, and, in addition, one was obtained from New Hampshire and one from Winnipeg, Canada. About 45 inbred lines of corn and one or two lines of teosinte were inoculated with most of these collections.² As a result of the preliminary inoculations, 8 selfed lines of corn were selected as differential hosts.

The methods used in making inoculations were essentially the same as those described by Stakman and Piemeisel (10) in inoculating cereals and grasses with *Puccinia graminis*.

The method of indicating the degree of infection was adapted somewhat from that used by Stakman and Levine (11) in their work with *P. graminis tritici*. However, the types of infection produced by *P. sorghi* were neither as distinct nor as consistent as those caused by *P. graminis*. Therefore it was necessary to modify their system somewhat. In table 3 the numbers are used to indicate the size of pustules as follows:

² Most of the seed of the selfed lines of corn used in the greenhouse experiments was furnished by the Section of Plant Genetics, University of Minnesota. Some was obtained from Dr. R. J. Garber, West Virginia Agr. Exp. Sta. Seed of teosinte was furnished by Mr. F. D. Richey, U. S. Department of Agriculture.

- 1—Uredinia very small, rather scattered, and not confluent.
- 2—Uredinia somewhat larger than in 1, usually scattered, and not confluent, except in rare cases.
- 3—Uredinia moderately large, seldom confluent.
- 4—Uredinia large and frequently confluent.

Plus or minus signs after the figure indicating the size of pustules means plus or minus fluctuation within the class. For example, 1 – means that the pustules are extremely small, even for class 1.

The letter n following the figure indicates the presence of necrotic areas.

Plus and minus signs after the n indicate the degree of necrosis. For instance, n indicates moderate necrosis, n – rather indistinct necrosis, and n + very decided necrosis. There appear to be practically all combinations of pustule size and necrosis.

Results

It is evident that there are at least seven distinct physiologic forms which can be recognized readily by their effect on eight selfed lines of corn (table 3). The differences in the infection capabilities are shown more clearly in the following analytical key:

ANALYTICAL KEY FOR IDENTIFICATION OF PHYSIOLOGIC FORMS OF *Puccinia sorghi*

Golden Bantam, culture 1-S, very resistant	Form 1
Golden Bantam, culture 1-S, very susceptible	
Longfellow, culture 611-A, very resistant	
Minnesota No. 13, culture 213, very resistant	Form 2
Minnesota No. 13, culture 213, very susceptible	
Rustler, culture 396, resistant	Form 3
Rustler, culture 396, susceptible	Form 4
Longfellow, culture 611-A, susceptible	
Minnesota No. 13, culture 213, resistant	Form 5
Minnesota No. 13, culture 213, susceptible	
Northwestern Dent, culture 507, resistant	Form 6
Northwestern Dent, culture 507, susceptible	Form 7

Form 1, isolated from a collection obtained from San Antonio, Texas, was by far the weakest in pathogenicity. In fact, it was so weak that it was rather difficult to keep it in culture. This form did not develop normally even on culture 1-S, a strain of Golden Bantam, which was very susceptible to 17 of the rust cultures obtained. At least 22 lines of corn were inoculated with form 1, and not a single one was susceptible. As a rule it is relatively easy to maintain and propagate corn rust in the greenhouse, but form 1 was lost before a congenial host could be discovered. In addition to differences in pathogenicity, form 1 also differed in appearance from the other six forms. The color of the uredinia was much lighter than that

TABLE 3.—*Reaction of eight selfed lines of corn to seven physiologic forms of corn rust.*

Form no.	Place of collection	Lines of corn and rust reaction							
		Golden Bantam, culture 1-8	Eldridge, culture 3-5-2-2-1-1	Minn. No. 13, culture 212	Minn. No. 13, culture 213-A	Rustler, culture 396	Rustler, culture 410	North-western Dent, culture 507	Longfellow, culture 611-A
1	San Antonio, Texas	1-n+	0n+ to 1n-	3=
2	Stillwater, Oklahoma	4+n-	0n+ to 1n	2 to 3n-	1n+	3+n	2+n	3n	1+n+
3	Concordia, Kansas	4-n	0n+ to 1n	1n+ to 2+n	3=	2+n	2n-	3	0n+ to 1n+
4	University Farm, Minnesota	4-n-	1n+ to 2+n	3+	4n+	2n-	3+	0n+ to 1n+
5	Lincoln, Nebraska	4+n-	0n+	3+n	1n+	4-n	4-	3n-	3=n
6	Iowa	4n-	2n-	3+n	3±n	3-	2-	3n
7	Hutchinson, Minn.	4n	0n+ to 3n	3+	3n	1n+ to 3n	3-	3+	3n

of the other forms, and under the microscope the spores also were much lighter in color than those of the other forms. It is barely possible that this difference in color may have been due to the fact that the uredinia of form 1 never were produced on a congenial host, but this seems unlikely. Form 1 also differed from all of the others in the fact that it never produced any teliospores, although other forms in neighboring booths in the greenhouse produced them in abundance on certain hosts. Wellensiek (13) observed that the formation of teliospores often was induced by the resistance of the host, but this was not true of form 1.

There may be a number of physiologic forms in the same general locality. Two forms, numbers 4 and 7, were isolated from material collected in Minnesota, and there is some evidence that there is a third form in the State. Forms 4 and 7 were obtained from corn grown on University Farm, St. Paul; while form 7 was obtained also from field corn near Hutchinson, Minn., about 60 miles west of St. Paul, and from three other localities in Minnesota, as well as Henniker, New Hampshire. It seems quite likely, therefore, that it may be widely distributed.

It is quite likely that there are numerous other physiological forms which could be recognized readily if more collections were made and more differential hosts inoculated. Because of the fact that selfed lines of corn may be heterozygous for rust reaction, it would be rather difficult to place the identification of forms of *P. sorghi* on as definite a basis as that of some of the cereal rusts.

DISCUSSION AND CONCLUSIONS

By far the best method of reducing losses from certain corn diseases is the development of disease-resistant lines. However, it already has been shown (1) that there are numerous physiologic forms of the corn smut fungus, *Ustilago zeae*. It has been shown in this paper, as well as by the work of Mains, that there also are physiologic forms of *P. sorghi*. It is imperative, therefore, to take these facts into consideration in any program for the development of smut- and rust-resistant lines of corn.

The facts brought out in this paper show clearly that a pathogene such as *P. sorghi*, which is relatively innocuous to most varieties of corn now grown commonly, may be, under certain conditions, very destructive to selfed lines which are produced for the purpose of general varietal improvement or for resistance to certain diseases. It is important to realize that in producing selfed lines of corn one is in reality producing "physiologic forms" of a host plant and that these forms may be extremely susceptible to a pathogene hitherto considered of little economic importance.

It requires several years of continuous self-fertilization to develop inbred lines of corn which are relatively homozygous. It is the common practice

of corn breeders during this period of continuous inbreeding to select rigidly for freedom from striking abnormalities and, in some cases, susceptibility to various diseases. Strains which are highly resistant to at least some physiologic forms of *U. zeae* have been obtained as a result of such selections. Rust appears to be somewhat less important than smut, yet it is quite destructive to certain selfed lines of corn. While it would be desirable to ascertain the reaction of selfed lines to the different physiologic forms of *P. sorghi* present in the locality, it seems probable that, by careful selection for resistance to rust during the several years of selection in self-fertilized lines, and by close observation of the lines selected for use in the production of crosses or synthesized varieties, those biotypes which are highly susceptible to the rust forms present can be eliminated with a fair degree of success. The facts presented indicate the necessity of careful selection for rust resistance either under artificially induced or natural epidemic conditions so that any new varieties produced will be at least as resistant as was the original variety from which the inbred lines were developed.

SUMMARY

1. *Puccinia sorghi* Schw. may become a destructive pathogene on newly developed lines of corn. It caused considerable damage to certain inbred lines of corn grown at University Farm, St. Paul, in 1923 and in 1925.

2. Notes were taken on the rust and smut reaction of 171 selfed lines of corn. Some lines were extremely susceptible, others highly resistant, while still others were intermediate in their reaction to rust.

3. All combinations of resistance and susceptibility to corn rust and to corn smut appeared in the field.

4. Collections of *P. sorghi* were obtained from many different places in the United States and one place in Canada. Forty-five selfed lines of corn were inoculated with most of these collections.

5. Seven physiologic forms of *P. sorghi* can be recognized by their parasitic behavior on eight selfed lines of corn. Two forms were isolated from Minnesota, and one each from the following states: Texas, Oklahoma, Kansas, Nebraska, and Iowa.

6. Form 1, besides being relatively weak in pathogenicity, also produced uredinia decidedly lighter in color than those of the other six physiologic forms. It never produced telia, while other forms produced them readily.

7. The fact that there are numerous physiologic forms of *P. sorghi*, some of which are quite injurious to certain selfed lines of corn, should be taken into consideration in breeding work.

8. It is important in producing new varieties of crop plants to determine their reaction to physiologic forms of the most important pathogenes

and to take cognizance of the fact that new varieties or lines may be far more susceptible to hitherto unimportant pathogenes than varieties now commonly grown.

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THE FUNGICIDAL ACTION OF LIQUID LIME SULFUR

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Lime-sulfur solutions have been known and used as fungicides for a long time. Lodeman (5, p. 16) reports their use as early as 1852. It was not until 1906, however, that this fungicide and insecticide came into extensive use in the United States. At that time Cordley (1), in Oregon, introduced the material for fungicidal and insecticidal purposes.

The fungicidal properties of lime-sulfur solutions have been a topic of wide interest. It has been the opinion of most investigators that the fungicidal value of liquid lime-sulfur is indirect, and that finely divided sulfur or some oxidation product of the same, which is liberated on exposure to the oxygen of the air, is the toxic substance.

Foreman (4), in 1910, ascribed the fungicidal properties of liquid lime-sulfur to the alkalinity expressed by such solutions. Eyre and Salmon (2), in 1916, reported the results of investigations on the fungicidal properties of spray liquids. During this work they found that the fungicidal properties of lime-sulfur solutions were entirely a function of the soluble polysulfide content. They proved to their satisfaction that the alkalinity of the solutions was not responsible for their fungicidal properties. They compared, quite exhaustively, the effects of various sulfide mixtures. Their criterion of toxicity was based upon the effect of the solutions upon the growth of superficial patches of the mycelium, conidiophores, and conidia of the powdery mildew fungus, *Sphaerotheca humuli* (D. C.) Burr. They made a point of the fact that only patches of the same age could be used. Eyre, Salmon, and Wormald (3), in 1919, continuing the experiments on lime-sulfur, again came to the conclusion that the fungicidal properties of this material were due to the amounts of sulfides in solution. They found that sodium thiosulphate was ineffective in controlling the fungus. During this work, they found that one of their sulfide solutions which contained a great deal of finely divided sulfur, indicating the breaking down of the sulfide, was just as effective as one in which the sulfide had not been thus affected. As the content of soluble sulfide had been reduced beyond that which was found to be effective, they attributed the toxicity of the solution containing sulfur to both constituents. They left the subject of the toxic properties of lime-sulfur solutions still in doubt.

In 1922, Young (7) published on the toxic properties of sulfur. Quoting many researches and advancing considerable data himself, he was of the

¹ Contribution from the Division of Plant Pathology, University of California.

opinion that a relation existed between toxicity and an oxidation product of sulfur, namely, pentathionic acid. He regarded the effect of liquid lime-sulfur as being due to the oxidation of the sulfides to finely divided sulfur, which is, in turn, oxidized to some higher form which acts as the toxic substance. He found that calcium thiosulphate had no value as a fungicide, thereby supporting Eyre in this respect. He did not regard the sulfide ion as the toxic substance, but based his whole theory upon the oxidation products of elemental sulfur.

In England, during the meeting of the Economic Botanists in 1926, the matter of the toxic effect of sulfur was thoroughly discussed at a symposium on the subject (6). The fungicidal properties of the polysulfide solution were attributed to the direct action of sulfur which is rapidly formed by the decomposition of the sulfide in the presence of oxygen. This toxicity, it was claimed, could be shown by bringing plants infected with mildew into an atmosphere which contained heated sulfur. If the finely divided sulfur had been screened from the chamber toxicity was not demonstrated. The hypothesis of Young was held untenable on account of the fact that toxicity is demonstrated at hydrogen ion concentrations which are inhibitive to the formation of pentathionic acid.

EXPERIMENTAL DATA

In connection with laboratory studies on the peach rust fungus, *Tranzschelia punctata* (Pers.) Arth., a variety of fungicides was tested. Liquid lime-sulfur solutions, diluted as high as one to a hundred, gave excellent results.

During observation with the microscope of germinating urediniospores which had been sprayed with liquid lime-sulfur solutions, it was noticed that the lumina of the germ tubes became more or less filled instantaneously with what appeared to be sulfur globules, which resembled those appearing upon the slides from the oxidation of the sulfide solutions. Many observations were made, always with the same results. Whenever the germ tubes came in contact with the solution, the globules were formed. This is shown quite well in Plate IV, A. The germ tube can be seen in an area which formerly was a drop of lime-sulfur solution, where sulfide has changed to sulfur globules. Within the germ tube similar globules are seen, especially near the lime-sulfur drop. At this stage it is difficult to differentiate between the globules within the germ tubes and those on the slide. The question which naturally arose was, were these globules within the tubes or were they on the surface of the spores and tubes. It became necessary to demonstrate in some manner the actual presence of the globules within the tubes and to ascertain if they were, as the writer suspected, sulfur globules. That this is the case was proved by the following method.

In some preliminary studies, it was found that the urediniospores of this fungus would not germinate in drops of tap or distilled water. It was noted also that spores which were scraped off of the sori would not germinate. Only those which were shaken off germinated readily. From all indications it was purely a matter of maturity, those spores which were easily shaken from the sori being the mature ones. The spores germinated only in an atmosphere which was saturated. Accordingly, the method used for the germination of the spores was as follows: the spores were dusted or shaken on to slides; the slides containing the spores were then placed in a saturated atmosphere at room temperature. In 12 hours the germ tubes reached their maximum growth, at which time the germinated and ungerminated spores were sprayed with the desired strength of lime-sulfur solution. This was accomplished by spraying with an atomizer.

After considerable effort, the problem of dissolving away the sulfur on the slides without interfering with that within the germ tubes was solved. The method is quite simple, once worked out. The steps in the method are as follows. The slide is washed with dilute hydrochloric acid to remove the carbonates and thiosulphates which, as well as sulfur, remain upon the slide. After the treated slide has been washed with the acid, it is cleaned off with distilled water. The slide is dried by slightly warming in a flame, and, while warm, flooded with carbon tetrachloride; this dissolves the sulfur which remains on the slide. For some reason, possibly because the sulfur inside the tubes and spores is fixed within a protoplasmic mass or is surrounded by water, the tetrachloride does not penetrate into these parts. The sulfur of the outside system is removed by this method. Flooding a few times removes all of the sulfur from the slide and surfaces of the spores and tubes. After the tetrachloride has evaporated, a few drops of sodium hydroxide, N/5 solution, are placed on the slide. The hydroxide solution appears to penetrate into the cells and slowly reduces the elemental sulfur to sulfide ion. The sulfide ion can then be demonstrated by placing a few drops of sodium nitroprusside along with the hydroxide. The globules within the tubes and spores are now a deep violet color, which is indicative of the exchange of the NO part of the nitroprusside ion with the sulfide ion from the globules. The plastic globules are in this manner shown to be elemental sulfur. Plate IV, B shows the globules after having been treated with the hydroxide and nitroprusside ion. The sulfur within the spores does not show very well. Under the microscope, however, these are deep violet in color.

The same technique is as satisfactory for sprayed spores. Spores and germinating spores which have not been sprayed and which do not contain the globules do not give the nitroprusside reaction when hydroxide is allowed to diffuse into the cells. Liquid lime-sulfur, when allowed to dry

with the resultant liberation of plastic sulfur, reacts, giving the same violet color as obtained within the tubes. Finely divided elemental sulfur, when acted upon by strong sodium hydroxide solutions, gives the same reaction with nitroprusside.

It is evident from this demonstration that the globules within the germ tubes and spores are plastic sulfur. By the presence of sulfur within the spores and tubes one comes to the conclusion that the sulfide which had been sprayed on them had entered the cell and had been oxidized to some form of elemental sulfur. The oxidation of sulfide to sulfur, probably by direct reduction of the protoplasm, apparently interferes with the growth of the germ tubes, and growth stops. Many observations with spores and germinated spores were made relative to this point. Table 1 shows the effect of liquid

TABLE 1.—*Effect of liquid lime-sulfur, 1-100, on the length of germ tubes of urediniospores of Tranzschelia punctata.*

Germ tube	Time sprayed	Length in microns after			
		3 hours	6 hours	9 hours	12 hours
1	Third hour	6	6	6	6
2	do	8	8	8	8
3	do	6	6	6	6
4	Sixth hour		17.5	17.5	17.5
5	do		23.0	23.0	23.0
6	do		15.0	15.0	15.0
7	Ninth hour			38.0	38.0
8	do			46.5	46.5
9	do			35.0	35.0
10	Not sprayed				61.5
11	do				82.0
12	do				77.0
13	do ^a				71.5

^a Average of 12 germ tubes.

lime-sulfur solution (diluted 1 part to 100 parts of water) on the length of the germ tube. Table 2 shows the effect of various sulfur compounds upon the germinability of urediniospores. Apparently the limit of laboratory control with liquid lime solutions lies near the dilution of 1-100. A few of the spores germinate at this dilution, while dilutions less than this give perfect results.

It is evident, from the data presented, that an injurious effect on the metabolism of the host results from the application of various solutions of liquid lime-sulfur. The formation of sulfur from sulfide brings about an irreversible change in the oxidation-reduction system of the cell. The in-

TABLE 2.—*Effect of sulfur compounds upon the germinability of urediniospores of Tranzschelia punctata*

Compound and dilution	Temperature	No. spores counted	No. spores germinating	Percentage of germination
Commercial dry lime sulfur 14 lbs.-100 gals.				
H ₂ O	22° C.	4036	242	6.0
Liquid lime-sulfur, 1-100...	do	1376	12	0.9
Liquid lime-sulfur, 1-50.....	do	3001	0	0.0
Liquid lime-sulfur, 1-10.....	do	3378	0	0.0
Powdered sulfur (old lot)	do	2060	198	9.1
Powdered sulfur (new lot)	do	8336	118	1.4
Sulfur paste, gas house residue	do	1723	49	2.8
No treatment	do	12,425	3516	28.3

jury to the system can be interpreted in the light of the oxidation of the sulfide to sulfur through the agencies of the cell.

DISCUSSION

The fungicidal properties of polysulfide solutions are dependent, at least in part, upon its soluble sulfide content. The theory of Young and others—that polysulfide solutions act only when the oxidation product of the resultant liberated sulfur is formed—seems unnecessary in view of the observations and data of the writer.

The fact that the formation of sulfur within the germ tubes represents an instantaneous reaction, and that the germ tubes and spores are arrested in their development, forces one to believe that the oxidation of the sulfide to sulfur is the lethal factor. In the light of the development of knowledge regarding the oxidation-reduction systems and of the hydrogen-ion relations of cells, one can better understand the effect of sulfide solutions upon the protoplasm of the germ tubes and spores. The absorption of sulfide solutions brings about at least two changes: (1) a reduction of the oxidation-reduction potential, and (2) of the hydrogen ion concentrations. Either one in itself, if carried far enough, would be responsible for an irreversible change in the poise of the protoplasmic system. It is hardly possible that the small amounts of sulfide solutions which were used could bring about such an irreversible change principally on account of hydrogen ion relations. It seems most probable that the poisoning material of the oxidation-reduction system has been used up to a point beyond which recovery is impossible. Since the formation of elemental sulfur is coincident with, or followed by, death of the organism, the logical conclusion is that this sub-

stance represents the end product of a reaction concerned with the oxidative properties of the protoplasm of the cell, probably influenced, in part but not entirely, by the alkalinity of the solution.

These results confirm those of Eyre and Salmon upon the effect of liquid lime-sulfur solutions—that the toxic properties of sulfide solutions are functions of their respective soluble sulfide content.

SUMMARY

1. Liquid lime-sulfur solutions are instantaneously reacted upon by the protoplasm of the urediniospores and germ tubes of the peach rust fungus, *Tranzschelia punctata* (Pers.) Arth.

2. Globules of plastic sulfur are formed within the lumina of the germ tubes and within the spore cover.

3. A method for the determination of plastic sulfur within the germ tubes and spores is presented.

4. The reduction of the protoplasm of the host, through the oxidation of polysulfide to elemental sulfur, appears to be an important factor in the fungicidal action of liquid lime-sulfur, as determined by its effect upon the growth of germ tubes and upon the germinability of spores.

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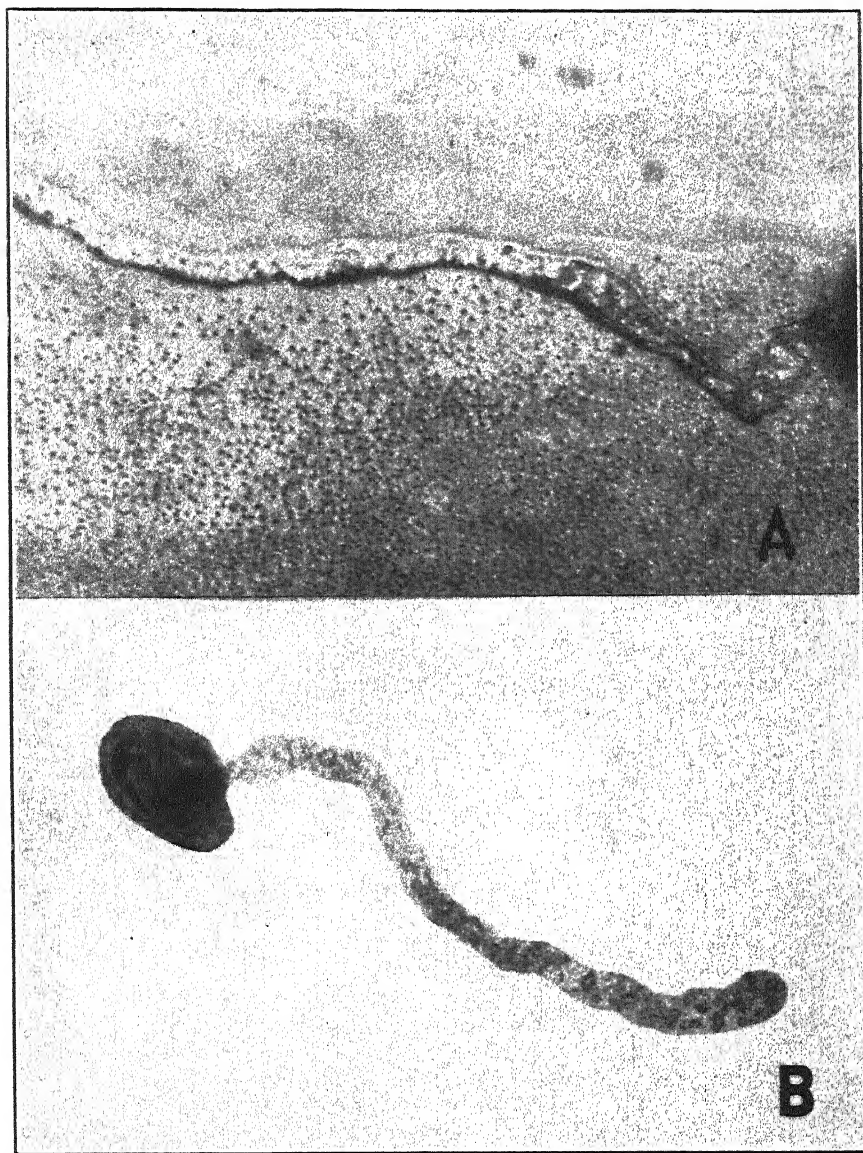
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EXPLANATION OF PLATE IV

A. Germ tube of urediniospore. Sprayed with lime sulfur 1-100 dilution and showing the sulfur globules on the slide and within the germ tube.

B. Germ tube of urediniospore. Sprayed with lime-sulfur 1-100 dilution and showing the sulfur globules within the germ tube after treatment with sodium hydroxide and nitroprusside ion. $\times 940$.



COMPARATIVE VIRULENCE OF CERTAIN STRAINS OF PYTHIUM IN DIRECT INOCULATION OF CONIFERS¹

E. J. ELIASON

INTRODUCTION

Many of the reports of the casual relationships between the different damping-off fungi and their hosts have been based upon the presence of fungi in diseased seedlings rather than upon inoculations with them. Hartley (4) demonstrated by inoculation that the various strains of the same species differed in their ability to cause damping-off of coniferous seedlings. Edson (1) demonstrated also that only part of the *Corticium vagum* strains isolated from sugar beets were able to attack that host vigorously. Gilbert (2) found by repeated inoculations that some strains of *Cladosporium cucumerinum* were uniformly virulent to cucumbers while others failed to produce infection. Because of this strain variability it is entirely possible that a saprophytic strain of a species which is usually parasitic may be associated with a damped-off seedling or that a parasitic strain of some damping-off fungus may be living as a saprophyte in a seedling damped-off by some other fungus. Therefore it is necessary to test the pathogenicity of damping-off fungi by inoculation experiments.

The object of the present investigation was to test the parasitism of certain *Pythium* strains and other fungi which had never before been tested on coniferous seedlings, and to determine their virulence in relation to one another and to other fungi used as standards by Rathbun-Gravatt (7). A list of the fungi tested is given in table 1.

¹ This article is a part of a thesis presented to the faculty of the New York State College of Forestry, Syracuse, N. Y., for the partial fulfillment of the requirements for the degree of Master of Science. The work herein reported was done in partial cooperation with the Office of Forest Pathology, United States Department of Agriculture, and is a continuation of some of the inoculation experiments which were started by that office. The *Pythium debaryanum* (²) cultures isolated from coniferous hosts were secured from that office. They are of the type formerly considered as *Pythium debaryanum* but in the present uncertain state of knowledge concerning this group it is possible that some of them belong to other species. The *Pythium* strains from angiospermous hosts were sent by Dr. Charles Drechsler, Office of Vegetable and Forage Diseases, U. S. Department of Agriculture. The culture of *Aphanomyces euteiches* was furnished by Dr. F. R. Jones, of Wisconsin. The writer is indebted to Mrs. A. R. Gravatt, Dr. Carl Hartley, and Dr. L. H. Pennington for their kindly criticisms and suggestions.

TABLE 1.—*Identity and source of the fungi tested*

Name	Strain no.	Source		
		Host	Locality	Isolator
<i>Alternaria</i> sp.		<i>Pinus resinosa</i>	New York	E. J. Eliason
<i>Aphanomyces euteiches</i>		<i>Psium sativum</i> (root)	Wisconsin	F. R. Jones
<i>Corticium vagum</i>	147 ^a	<i>Picea engelmannii</i>	Washington, D. C.	C. Hartley
<i>Fusarium moniliforme</i>	249 ^a	<i>Pinus ponderosa</i>	Kansas	T. C. Merrill
<i>Fusarium</i> sp.		<i>Pseudotsuga taxifolia</i>	New York	E. J. Eliason
<i>Pythium debaryanum</i>	296 ^b	<i>Beta vulgaris</i>	Wisconsin	H. A. Edson
<i>P. debaryanum</i> (?)	1036	<i>Pinus banksiana</i>	Nebraska	G. G. Hahn
do	1040	<i>Pinus sylvestris</i>	Virginia	do
do	1047	do	do	do
do	1071	do	do	A. R. Gravatt
do	1072	do	do	do
do	1074	do	do	do
do	1080	<i>Pinus taeda</i>	do	do
do	1084	<i>Pinus sylvestris</i>	do	do
do	1086	do	do	do
do	1091	<i>Pseudotsuga taxifolia</i>	Colorado	G. G. Hahn
do	1092	do	do	do
do	1093	do	do	do
do	1094	do	do	do
do	1095	do	do	do
<i>P. debaryanum</i>	1E (Jones no. 662) 1922	<i>Psium sativum</i> (root)	Wisconsin	F. R. Jones
<i>Pythium</i> sp.	2E	do	New York	C. Drechsler
do	3E (spiny)	do	Georgia ?	do
<i>P. aphanidermatum</i>	4E	<i>Nicotiana tabacum</i>	Africa	Dade-Buller
<i>Pythium</i> sp.	5E	<i>Cucumis sativa</i>	Illinois	
do	8E (Jones no. 830-1)	<i>Avena sativa</i> (root)	Wisconsin	F. R. Jones
<i>P. debaryanum</i>	9E	<i>Citrullus vulgaris</i>	Virginia	C. Drechsler

^a Used in inoculation experiments by Hartley et al (3, 4) and by Rathbun-Gravatt (7).

^b Used by Edson (1).

METHODS OF INOCULATION

In the writer's experiments three methods of direct inoculation were used, all of which have been described by Rathbun (6). Throughout this paper these methods will be designated as (a) "platform method," (b) "petri dish method 1," and (c) "petri dish method 2." The "platform method," in which the seedlings were inoculated about 2 cm. above the soil surface, was slightly modified: the seedlings were not grown in glass cupboards, and bell jars instead of individual celluloid cones were used as damp chambers. In the "petri dish method 1," sterile seedlings were placed in petri dishes, the bottoms of which had been covered with moist filter paper, and inoculum placed on any selected portion. In "petri dish method 2,"

TABLE 2.—Results of inoculating the stems of *Pinus resinosa* with four strains of *Pythium debaryanum* (?) by the platform method

Strain no.	No. seedlings inoculated	No. seedlings in contact with inoculum at end of experiment ^a	Seedlings damped-off		
			No.	Percentage of total	Percentage of those in contact
1091	40	20	7	18	35
1092	40	21	14	35	67
1093	40	27	4	10	15
1094	40	27	14	35	52
Control	100	56	0	0	0

^a Because of excessive drying of the sand the inoculum was not in contact with part of the seedlings at the end of the experiment.

TABLE 3.—Results of inoculating, by the platform method, stems of *Pseudotsuga taxifolia* with strains of *Pythium debaryanum* (?) reisolated from the *Pinus ponderosa* stems used in the previous experiment (table 2)

Strain no.	No. seedlings inoculated in each series	Seedlings damped-off			
		Series I		Series II	
		No.	Percentage	No.	Percentage
1091	20	0	0	0	0
1092	20	2	10	6	30
1093	20	1	5	1	5
1094	20	3	15	2	10
1095 ^a	20	3	15	1	5
Control	20	0	0	0	0

^a Original strain not used in previous experiments.

TABLE 4.—Results of inoculating *Pinus strobus* stems with the five original strains of *Pythium debaryanum* (?) and with other fungi by the platform method

Fungus	Strain no.	No. seedlings inoculated	Seedlings damped-off	
			No.	Percentage
<i>Aphanomyces euteiches</i>		8	0	0
<i>Corticium vagum</i>	147	7	5	71
<i>Fusarium moniliforme</i>	249	7	1	14
<i>Fusarium</i> sp.		8	0	0
<i>Pythium debaryanum</i> (?)	1091	8	1	12
do	1092	11	1	9
do	1093	8	4	50
do	1094	7	7	100
do	1095	12	0	0
Control		20	0	0

TABLE 5.—*Results of inoculating Pinus banksiana stems with three reisolated^a strains of Pythium debaryanum (?) by petri dish method 1*

Strain no.	No. seedlings inoculated	Seedlings damped-off	
		No.	Percentage
1091	10	2	20
1092	10	6	60
1093	10	4	40
Control	10	0	0

^a Same strains as referred to in table 3. Originally from *Pseudotsuga taxifolia* and reisolated from *Pinus resinosa*.

sterile medium was inoculated with the desired fungus, and after the mycelium had completely covered the surface, a cut was made across the center of the dish and all the material on one side of the cut removed. The root or stem of the seedling was then placed upon the medium remaining in the petri dish.

In each experiment, control plants were treated in exactly the same manner as the others except that sterile agar was used instead of agar inoculated with fungus. All the fungi were grown on cornmeal agar made according to the formula used by the Shear and Stevens (8) for *Endothia* cultures.

All the inoculations referred to in any one table were made at the same time. In the first experiment, of which the results are given in table 2, the temperature varied from 15° to 25° C. For all the others the temperature was kept fairly constant at about 21° C.

TABLE 6.—*The rank in virulence of five different strains of Pythium debaryanum (?) on four host plants. Summary of results of tables 2-5*

Strain no.	Rank in virulence on				
	<i>Pinus resinosa</i>	<i>Pseudotsuga taxifolia</i>		<i>Pinus strobus</i>	<i>Pinus banksiana</i>
		Series I	Series II		
1091	3	5.0	5.0	3	3
1092	1	3.0	1.0	4	1
1093	4	4.0	3.5	2	2
1094	2	1.5	2.0	1
1095	—	1.5	3.5	5

TABLE 7.—Results of inoculating *Pseudotsuga taxifolia* stems with strains of *Pythium debaryanum* (?) and with other organisms by the platform method

Fungus	Strain no.	No. seedlings inoculated	Seedlings damped-off	
			No.	Percentage
<i>Alternaria</i> sp.		16	0	0
<i>Aphanomyces euteiches</i>		14	2	14
<i>Corticium vagum</i>	147	19	5	26
<i>Fusarium</i> sp.		15	0	0
<i>Fusarium moniliforme</i>	249	14	4	29
<i>Pythium debaryanum</i> (?)	296	16	5	31
do	1036	16	4	25
do	1040	17	6	35
do	1047	16	1	6
do	1071	15	5	33
do	1072	17	0	0
do	1074	15	3	20
do	1080	16	7	44
do	1084	17	3	18
do	1086	14	0	0
do	1094	16	3	19
Control		16	0	0

EXPERIMENTAL RESULTS

The results of all of the experiments are given in tabular form (Tables 2 to 10). The data given in tables 2 to 5 inclusive are summarized in table 6.

TABLE 8.—Results of inoculating the stems of various coniferous hosts with various damping-off fungi by the platform method

Fungus	Host	No. seedlings inoculated	Seedlings damped-off	
			No.	Percentage
<i>Alternaria</i> sp.	<i>Pseudotsuga taxifolia</i>	17	0	0
<i>Aphanomyces euteiches</i>	do	17	2	12
<i>Corticium vagum</i>	<i>Pinus banksiana</i>	16	11	69
do	<i>Pseudotsuga taxifolia</i>	16	9	56
<i>Fusarium moniliforme</i> (249).....	do	16	0	0
do	<i>Pinus ponderosa</i>	6	0	0
<i>Pythium debaryanum</i> , No. 1094				
(Original)	<i>Pseudotsuga taxifolia</i>	17	0	0
Control		30	0	0

TABLE 9.—Results of inoculating roots of coniferous seedlings with *Aphanomyces euteiches* by the petri dish methods

Host	No. seedlings inoculated by each method	Seedlings with rotted roots			
		Petri dish method 1		Petri dish method 2	
		No.	Percentage	No.	Percentage
<i>Picea engelmanni</i>	5	3	60	2	40
<i>Pinus banksiana</i>	5	1	20	0	0
<i>P. ponderosa</i>	5	0	0	0	0
<i>P. strobus</i>	5	0	0	0	0
<i>Pseudotsuga taxifolia</i>	5	0	0	0	0
Control	5	0	0	0	0

TABLE 10.—Results of inoculating, by the platform method, stems of *Pseudotsuga taxifolia* with strains and species of *Pythium* isolated from angiospermous hosts

Fungus	Strain no.	No. seedlings inoculated	Seedlings damped-off	
			No.	Percentage
<i>Pythium debaryanum</i> (?)	1E	11	2	18
<i>Pythium</i> sp.	2E	9	4	44
do	3E	10	2	20
<i>P. aphanidermatum</i> ..	4E	11	3	27
<i>Pythium</i> sp.	5E	9	1	11
do	8E	12	3	25
<i>P. debaryanum</i>	9E	12	5	42
Control		25	1	4

SUMMARY

1. The experimental data show that, of the 22 species and strains of *Pythium* tested, all but three caused damping-off in some coniferous seedlings.

2. *Aphanomyces euteiches*, a parasite upon roots of *Pisum sativum*, caused damping-off and root rot in a few coniferous seedlings.

3. An *Alternaria* and a *Fusarium* isolated from coniferous material gave no evidence of parasitism in these experiments.

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THE RELATION OF TEMPERATURE DURING THE GROWING SEASON IN THE SPRING WHEAT AREA OF THE UNITED STATES TO THE OCCURRENCE OF STEM RUST EPIDEMICS

E. C. STAKMAN AND E. B. LAMBERT¹

Black stem rust is one of the typically epidemic diseases of plants. In the United States, the greatest variation in the severity of attack is in the upper Mississippi Valley, particularly in the area growing hard red spring wheat. In some years the rust causes relatively little damage; in others only a moderate amount; whereas in still others it develops devastating epidemics which must be experienced to be appreciated. The apparent suddenness of onset and the amazing rapidity of spread have driven many farmers into bankruptcy and many pathologists well nigh to distraction.

Obviously, several factors must operate in proper sequence and conjunction in order that an epidemic may develop. First of all, there must be abundant inoculum, favorable conditions for its dissemination and the germination of the spores, the entrance of the germ tubes into the plants, the subsequent rapid development within the plants, and the production of still more inoculum. There also must be a fairly dense and widespread population of susceptible hosts.

The first of the requirements usually is satisfied because the number of spores can multiply so rapidly. There are two sources of initial inoculum in the hard red spring wheat area: (1) aeciospores from the common barberry, and (2) urediniospores which may be blown in from the South. There usually is plenty of wind to disseminate the spores. Except in unusually dry years, dews are likely to be sufficiently heavy and frequent, even in the absence of frequent rains, to promote germination of the spores and entrance of the germ tubes; there are many susceptible varieties of small grains and wild grasses; there is practically always sufficient light for the development of the rust; but the temperature varies considerably in different seasons.

It has been shown by many observations and controlled experiments that light and temperature affect the development of the rust on inoculated

¹ Cooperative investigations between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Minnesota Agricultural Experiment Station.

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plants more than any other environmental factors. Predisposition of the host does not seem to be very important, as plants growing under fairly normal conditions are sufficiently susceptible to enable the rust to develop well.

The amount of sunlight, of course, varies in different years, but usually not enough to affect very profoundly the development of rust. This leaves temperature as one of the important variables in the environment which determine whether or not epidemics will develop.

Opinions regarding the effect of temperature on the development of rust have differed considerably. The controlled experiments of Melhus, Durrell, and Kirby (4), Stakman and Levine (7), Peltier (6), and Johnson (3) have given us pertinent facts. They indicate a profound influence of temperature on the germination of teliospores, the germination and longevity of urediniospores, and the rate of development of the uredinial stage. But the results of these studies on stem rust can not directly explain the development of epidemics. The rate of development of the host and the factors affecting it must be considered also, because in epidemic years there is a race between the pathogene and the host plant. One would expect the development of epidemics to be favored by cool weather because of the delayed maturity of the host. Freeman and Johnson (2) pointed out that such conditions favored the development of the epidemic in 1904. However, Blair (1) has shown that yields usually are higher in cool seasons than in hot ones. Therefore, it seemed desirable to determine whether there is a correlation between temperature during the growing season and the development of rust epidemics.

The historical method, so successfully used by Martin (5) in studying the relation of weather to epidemics of late blight of potato in New Jersey, also was used in the present study of the effect of temperature on the development of stem rust.

The average temperature prevalent during the growing season in epidemic years is compared with that for non-epidemic years from 1904 to 1925, inclusive. Barberries usually become rusted in May. The late spring and early summer months seem to be the critical time for the development of rust in the hard red spring wheat area. This period involves the development of the barberry leaves, the germination of teliospores, the dissemination of sporidia, the infection of the barberry, subsequent discharge of the aeciospores, and the beginning of local epidemics near barberry bushes. Spring wheat usually is sown in April, and its development is considerably affected by temperatures during May. During May, June, and July, the rust usually spreads from the barberries and multiplies in the uredinial stage. Wheat also is making its principal growth during June and July. After the first week in August, wheat and other small grains

usually are mature or so nearly so that rust does not develop on them. It is possible also that the temperature during the fall and winter may affect the development of rust by influencing overwintering of the uredinal stage and the production and maturity of teliospores. However, the factors affecting the production and maturation of teliospores are obscure, and the uredinal stage of the rust seldom, if ever, overwinters to any considerable extent in the spring wheat area. May, June, and July therefore were selected for study.

EPIDEMIC, INTERMEDIATE, AND NON-EPIDEMIC YEARS

There have been at least six destructive epidemics in the hard red spring wheat area during the past quarter of a century. Personal observations and the records of the U. S. Plant Disease Survey from 1904 to 1925 indicate that the worst epidemics occurred in 1904, 1911, 1916, 1919, 1920, and 1923. The following could be classed as intermediate years: 1905, 1906, 1908, 1914, 1917, 1921, 1922, and 1925. There was comparatively little rust in 1907, 1909, 1910, 1912, 1913, 1915, 1918, and 1924. The first series will be designated epidemic years, the second intermediate, and the third non-epidemic.

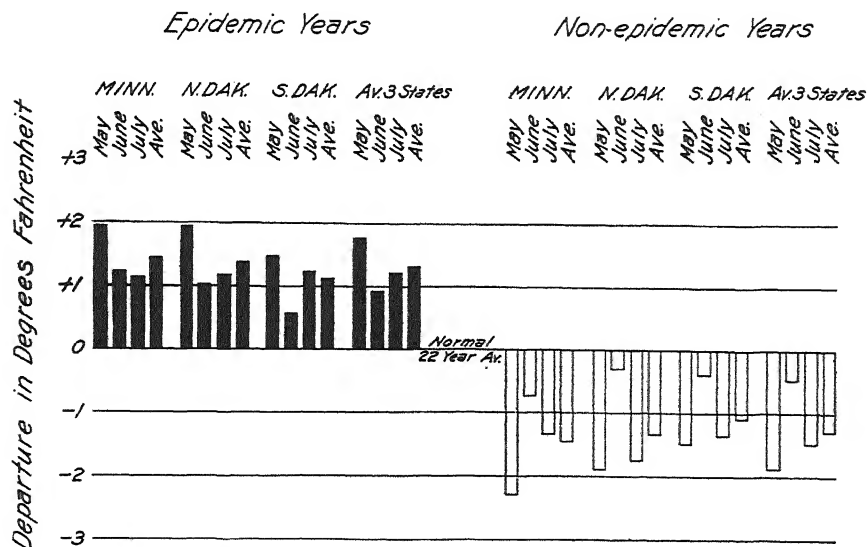


FIG. 1. A comparison of the departure from the normal of the average of the mean monthly temperature of all the epidemic years, with the average of all the non-epidemic years, for Minnesota, North Dakota, and South Dakota, in May, June, and July, from 1904 to 1925, inclusive.

TEMPERATURE AND EPIDEMICS

The average mean monthly temperatures during May, June, and July in Minnesota, North Dakota, and South Dakota for the epidemic years was compared with that for the non-epidemic years. As shown in figure 1, the average temperature throughout the growing season in all three states was consistently above normal in the epidemic years and below normal in the non-epidemic years.

The amount of departure from the normal for the 22-year period also was studied. As shown in figure 2, the three coolest years were non-epidemic years and the five warmest years were either epidemic or intermediate years. When the epidemic and non-epidemic years are considered separately, as in figure 3, it becomes apparent that destructive epidemics did not develop in seasons during which the average temperature was below 61° F. Nor were there any non-epidemic seasons when the average tem-

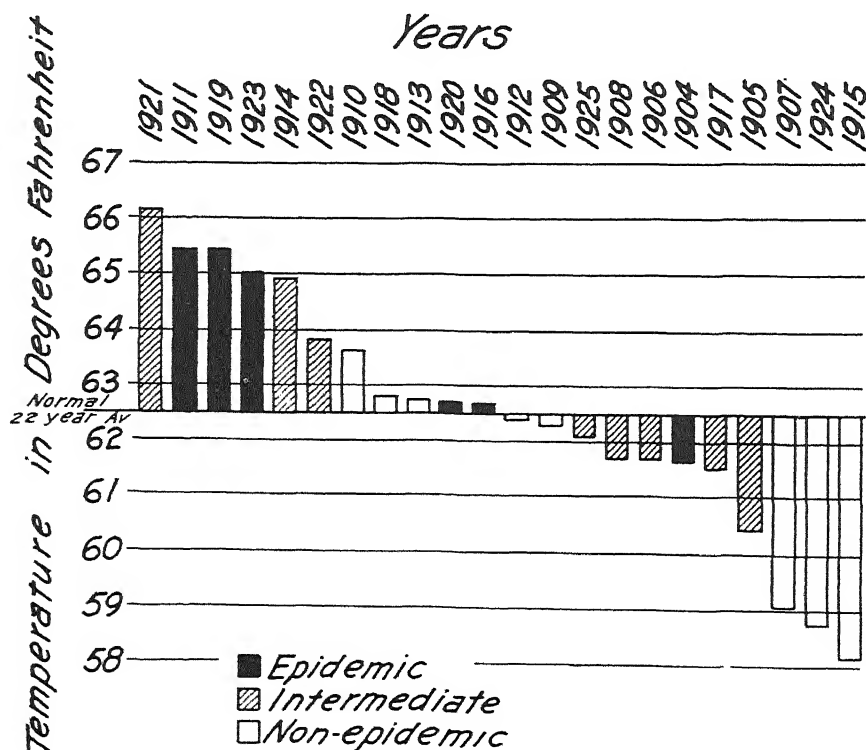


FIG. 2. A comparison of the departure from the normal of the average of the mean monthly temperatures for the growing season (May, June, and July) in the spring wheat area (Minnesota, North Dakota, and South Dakota) from 1904 to 1925, inclusive.

perature was above 64° F. There were both epidemic and non-epidemic years when the average temperatures were intermediate (61–64° F.). This is exactly what one would expect on the assumption that temperature is only one of many interdependent factors influencing the development of epidemics.

The weather conditions in three of the years with moderate temperatures are especially interesting: in 1910, because so little rust developed in spite of a comparatively high average temperature; and in 1916 and 1904, because very destructive epidemics developed when the temperature was moderate. In 1910, precipitation clearly was the limiting factor. The average precipitation was less than two inches for each of the three months under consideration in each of the three states, with the exception of June in South Dakota, when there was an average precipitation of 2.27 inches. There was such a decided and protracted drought that infection was practically impossible. It was the only year out of 19 when at least a moder-

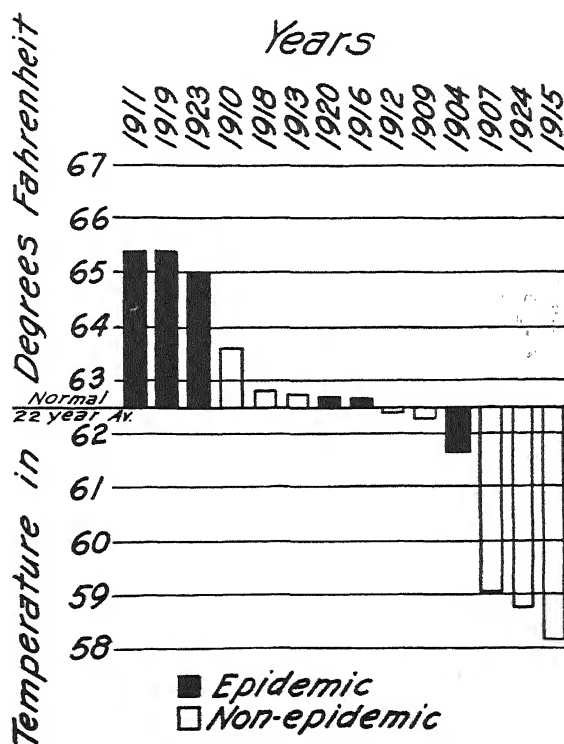


FIG. 3. A comparison of the departure from the normal of the averages of the mean monthly temperatures for the growing season (May, June, and July) in the spring wheat area (Minnesota, North Dakota, and South Dakota) which occurred during the stem rust epidemic years and non-epidemic years from 1904 to 1925, inclusive.

ately severe epidemic did not develop in the artificially inoculated rust nursery at University Farm, St. Paul, Minnesota. The epidemic of 1916 was the most destructive on record. This may seem surprising when one notes that the average temperature was but little above normal. But the average temperature during July was higher in all three states than that of any other year under consideration. Not only did the intense heat aggravate the damage from rust, but it undoubtedly was directly responsible for much of the injury popularly attributed to rust.

It is difficult to account for the epidemic of 1904. The temperature was lower than normal and the rainfall was not excessive. It is evident, therefore, that epidemics may develop even in moderately cool seasons, although this seems to be the exception rather than the rule.

CONCLUSIONS

From the data presented, it is evident that, in the past, there has been a tendency for destructive epidemics to develop in warm growing seasons, and for cool seasons to be comparatively free from rust. The amount of departure from normal also seems to have been correlated with the occurrence of epidemics. It is possible that these tendencies were coincidences, but it seems more reasonable to suppose that they represent a causal relationship and are indicative of the effect of temperature on the development of epidemics. The purpose of this paper is to point out this relationship.

The graphs presented are based on more detailed information, which will be given in a later paper together with a report of similar studies on the relation of other meteorological factors to the development of stem rust.

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STUDIES OF THE ASCIGEROUS STAGE OF *VENTURIA* *INAEQUALIS* (CKE.) WINT. IN RELATION TO CERTAIN FACTORS OF THE ENVIRONMENT¹

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The recent work of Keitt and Jones (8) has shown that, under conditions where ascospores are the only important source of inoculum for primary infection of the apple by *Venturia inaequalis* (Cke.) Wint., the presence of an abundant and timely ascosporic inoculum is one of the chief requisites for the development of epidemic outbreaks of the scab disease. Since the ascospores comprise the only important primary inoculum known in most apple-growing sections, any practical means of limiting their timely production and discharge would be of great potential value in the economic control of the disease. Inasmuch as the work hitherto done on apple scab has been concerned chiefly with the parasitic phase of the pathogen and with the development and control of the disease, it seemed desirable to give special attention in the present work to the saprophytic phase of the fungus in relation to some of the more important factors which influence its development. It is hoped that a more adequate understanding of the development of the ascigerous stage of the parasite may be of value in furthering the understanding of the epidemiology and control of the disease.

For a more general discussion of apple scab problems and for citations to the literature of the disease, reference may be made to the paper of Keitt and Jones (8) and the works cited therein.

RELATION OF LEAF INFECTION TO THE PRODUCTION OF THE ASCIGEROUS STAGE

In studying the quantitative aspects of the production of ascosporic inoculum, one of the first points for consideration is the relation of leaf

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infection to the production of perithecia. The following questions seemed worthy of consideration: Do the type and abundance of leaf lesions influence the production of perithecia? Does the mycelium of the fungus ramify the tissues of the dead leaves extensively and produce perithecia at considerable distances from lesions? Does the fungus spread from dead leaves which have been infected by scab into those which have not been infected and there produce perithecia?

Type of Lesion

It has long been observed that two general types of scab lesion occur on the leaves of the apple. The more conspicuous is the rather definitely margined type, which occurs very generally on the upper (ventral) surface of the leaf. Less conspicuous is the indefinitely margined type, which is found very commonly on the lower (dorsal) surface. There are gradations between these two types and neither is restricted to one surface of the leaf. Aderhold (1), Clinton (2), and Wiltshire (16) found that the younger leaves of the apple were more susceptible to infection by *V. inaequalis* than older leaves. Keitt and Jones (8) found that the upper (ventral) surface of the apple leaf becomes resistant more rapidly than the lower, and that the period of incubation is lengthened as resistance increases. The lesions which occur after considerable resistance has been developed, particularly those on the lower surface, are commonly diffuse and inconspicuous. The present writer has noted that, under field conditions, the majority of the lesions which appear during the latter part of the growing season are of the diffuse or indefinitely margined type.

Considerable practical importance attaches to the relation of the two types of lesions to the production of perithecia. Lawrence (11) reports that lesions which appear late in the season and produce few conidia produce perithecia in greater numbers than those which had borne conidia in large numbers. Killian (10) states that the cells at the center of the lesions die, and penetration of the inner tissues of the leaf takes place only at the periphery. The present writer has compared the two types of lesion in this regard in leaves cleared by the chloral hydrate-potassium hydroxide method (Peace, 12). Perithecia were produced commonly only beneath the periphery of the more definitely margined lesions, while they were produced in any part of the area beneath the diffuse type. It was also noted that the cells of the stromatoid layer near the center of the more definite type of lesion were frequently without protoplasmic contents, while the cells at the periphery were more or less filled with protoplasm. Comparative studies showed that the number of points of penetration of the leaf lamina by hyphae of the fungus seemed to bear a direct relation to the presence of

protoplasmic contents in the cells of the stromatoid layer adjacent to the points where the hyphae originated. There also appeared to be an inverse relationship of the number of conidiophores produced on a given area of the stromatoid layer to the presence of protoplasm in the fungal cells of that area. All parts of the stromatoid layer of the diffuse type of lesion had cells generally well filled with protoplasm. Furthermore, fewer conidiophores were usually found on this type of lesion than on the more definitely margined type. It would seem, therefore, that the diffuse type is the more important in relation to the production of perithecia, because (a) of its common occurrence in larger numbers and (b) of the possibility of production of ascocarps at any point beneath the lesion.

Spread of the Fungus in the Interior of Infected Leaves and the Relation of Number of Lesions to the Abundance of Perithecia

The extent of ramification and saprophytic development of the mycelium of the fungus in the dead leaves and the relation of abundance of lesions to the development of perithecia are of much importance to an understanding of the quantitative aspects of the development of the ascospore inoculum. A perusal of the literature gives the impression that the perfect stage develops as the result of an extensive saprophytic growth of the fungus in the dead leaves, with the suggestion that the amount of parasitic development on the leaf is not of major importance. The observations just reported do not conform with this conception. To gain further evidence on this question, leaves which bore from one to few lesions were gathered, outlined on paper with the aim of recording the exact location and size of each lesion, and overwintered in the orchard. In the spring these leaves were brought into the laboratory and placed in a moist chamber at 16° C. In a few days, as the perithecia came to maturity, it was found that they were present in the areas covered by the lesions, but in most cases could not be found farther than one centimeter from the margins of the originally observed lesions. In a few cases the perithecia were scattered over the surfaces of the leaves, regardless of proximity to the lesions which were visible when the experiment was started. Examination of cleared fragments of these leaves showed that the fungus was present over the areas occupied by these perithecia, as very sparse stromatoid layers spreading in a very narrow, fan-like arrangement beneath the cuticle of the leaf. Very few conidiophores arose from these lesions, which explains why they were not seen when the leaves were taken from the tree.

The presence of lesions which were not macroscopically visible when the leaves were green was brought to the attention of the writer in the autumn of 1926. On September 5 a survey of the University orchard showed that

only 9 per cent of the leaves of Virginia (crab) had visible lesions. Later in the autumn, after the leaves had turned yellow, it was noted that 80 per cent of the leaves of the same trees bore numerous inconspicuous lesions. Perithecia were produced in abundance from these lesions when the leaves were overwintered. The failure to observe inconspicuous or macroscopically invisible lesions may account for the prevalent conception that the fungus ramifies the laminae of the infected leaves extensively and produces perithecia in areas comparatively remote from the lesions.

Further studies of these cleared leaves failed to show any extensive spread of hyphae of the scab fungus in the laminae. They were abundant in the area beneath the lesions but did not extend very far in a lateral direction. A few measurements showed that, in some cases, perithecia were located at a distance of one millimeter from the nearest stromatoid layer, but very few were found farther away. It is probable that temperature and moisture may influence the extent of the ramification of the hyphae. These experiments were carried out during an autumn of frequent rains and moderate temperatures, conditions which were conducive to the growth of the hyphae in the laminae of the leaves. The relations of temperature and moisture to the development of the perithecia will be discussed later.

It would seem from these observations that, under ordinary autumn conditions in Wisconsin, the abundance of lesions influences, to a marked extent, the quantity of perithecia which will be produced in the overwintering leaves. More extensive experiments are necessary, however, to determine the extent of the spread of the fungus in infected leaves during autumns of excessive moisture and moderate temperatures.

Does the Fungus Invade Non-infected Leaves on the Ground?

If the fungus is able to spread extensively by means of conidia or mycelium to non-infected leaves on the ground, the presence of a few infected leaves among the clean leaves might be sufficient to lead to the production of an abundant supply of perithecia, regardless of the severity of leaf infection during the summer. This would, obviously, complicate a system of control which was aimed at reducing the primary inoculum by preventing infection of the living leaves.

Experiments were conducted in 1925-26 and 1926-27 in which uninfected apple leaves were overwintered in contact with heavily infected leaves. In another experiment of 1926-27, *Venturia*-free dead leaves were sprayed with a conidial and mycelial suspension of the scab organism and kept under conditions suitable for development of the fungus, after which they were placed in the orchard for over-wintering. Repeated examinations of sub-samples of these leaves which had been brought into the laboratory

in the spring and placed in a moist condition at 16° C. for several weeks failed to show the presence of perithecia in the leaves which were free from *Venturia* at the beginning of the experiment. The leaves which bore scab lesions developed numerous mature perithecia upon being kept for a few days under these conditions as controls. The uninfected leaves were in most cases much more disintegrated than the infected leaves.

RELATION OF THE TIME OF LEAF-FAIL TO THE DEVELOPMENT OF THE
ASCIGEROUS STAGE

Methods

Collections of scabbed apple leaves were made at various times during the autumn and early winter of three seasons. In 1924-25 the leaves were taken from the Virginia (crab) until this variety had become defoliated, after which collections were made from an unknown variety which retained its leaves until late in the winter. In 1925-26, parallel collections were made from the Virginia (crab) and the McIntosh varieties, while in 1926-27 parallel collections were made from the Virginia and the unknown variety mentioned above. These samples were placed in the orchard in open-meshed cloth bags made from curtain material. An abundance of sod provided an ideal location for overwintering the leaves.

In the more detailed studies of 1924-25, fixations of leaves from these collections were made at intervals during the autumn, winter, and early spring, and imbedded in paraffin. Flemming's medium and chromo-acetic fixing reagents were employed. These fixations provided the material for the earlier stages of perithecial development. Of necessity, a large amount of material was sectioned for study. The Pianeze III B stain (Vaughan, 14) was found to be excellent as a means of rapidly differentiating the interior of the perithecia when details were not necessary. For the more detailed studies Flemming's triple and Haidenhain's haematoxylin stains were used. Data were gathered on the diameters and stages of development of perithecia in each collection. The fixed and stained sections were studied carefully, and measurements were made of all perithecia of *V. inaequalis*. Care was taken that measurements were made only of sections through the center of the perithecia. While diameter was found to be a fairly good criterion of development of perithecia, especially in the early stages, the data on internal change give a better idea of the stage of advancement and are therefore shown in table 1. A difficulty in using average diameter of perithecia as a criterion of their stage of advancement lies in the fact that perithecial initiation continues over a considerable period. Consequently, the later formed perithecia would tend to mask the stage of advancement of those formed earlier. In order to minimize this

TABLE 1.—*Relation of time of leaf-fall to the development of perithecia of Venturia inaequalis, Madison, Wis., 1924-25*

Date of examination	Development ^a of perithecia in scabbed apple leaves placed on the ground at stated dates:								
	Aug. 12	Aug. 26	Sept. 9	Sept. 23	Oct. 7	Oct. 31	Nov. 10	Dec. 17	Feb. 9
Oct. 16	B								
Oct. 29	C	B	B	B	A	B	A		
Nov. 17	C	C	C	B	B	B	B		
Dec. 15	C	C	C	B	B	C	C		
Jan. 20	C	C	C	C	B				
Feb. 12	D	D	C	C	C				
Mar. 18	E	E	E	D					
Apr. 9	F	F							O
Apr. 13	G	F							O
Apr. 19	G	G	E						
Apr. 22	H	G	E						
Apr. 26		H	F	E	D				
May 11			H	H	F		D		
May 25									
June 10					H	H	H	H	O

^a Stages of development: A = Simple coil; B = Ascogonial; C = Filamentous; D = Pre-ascus; E = Early ascus; F = Delimited spores; G = Olivaceous spores; H = Discharge.

source of confusion, the measurements were arranged in the order of magnitude, and all but the highest 25 per cent were discarded. The average measurements referred to, therefore, are representative of the more advanced perithecia.

For the studies of the later stages of perithecial development, subsamples were taken from the collections, and the more advanced perithecia were picked from the leaves and crushed on glass slides. Tests for discharge were made by placing the moistened leaves over glass slides.

The Typical Development of Perithecia

Detailed studies of the morphology and cytology of *V. inaequalis* were not undertaken by the writer. In tracing the relation of certain factors to the development of the ascocarps, however, it was necessary to follow the grosser changes which mark their development. Such studies served (a) to avoid confusion with other leaf-invading fungi, and (b) to record definitely certain well-defined stages in ascocarp development. The valuable contributions of Killian (10) and Frey (4) were very helpful guides in this work.

The possibility of mistaking perithecia of other fungi for those of *V. inaequalis* was minimized by becoming familiar with certain definite stages in the development of perithecia of the scab fungus. Furthermore, it was found that the connection of the perithecia with the subcuticular stromatoid layer was in many cases traceable. The hyphae of *V. inaequalis* penetrated but a short distance, and the perithecia were produced immediately beneath the epidermal layer. This was especially true in leaves which had been placed on the ground rather late in the autumn.

A description of perithecial development is included here with explanations of the terms used in table 1. Killian (10), who worked with the fungus in naturally infected dead leaves, states that after leaf-fall hyphae are sent into the interior of the leaf from the stromatoid layer. He figures a hypha thus arising but does not show the details of the penetration into the interior of the leaf. The present writer has found that a very common mode of entry of the hyphae is between the walls of adjacent epidermal cells (Figs. 1-2). A single hypha may push between epidermal cells and gain entrance to the interior, or the walls may be pushed apart by the development of a group of fungal cells (Fig. 2). In many instances there was noted a wedge-shaped outgrowth of a cell of the stromatoid layer which had grown between adjacent epidermal cells and pried the walls apart. Probably such outgrowths gave rise to the groups of cells which were found frequently to have developed between the walls of adjacent epidermal cells. The peculiar habit of the fungus in forming this plectenchymatous stroma-

toid tissue is very marked in some instances. Cases have been found in dead leaves in which a layer of such tissue had been formed between the end walls of the palisade cells and the adjacent epidermal walls, while other cases have been found in which this tissue had developed in the palisade region. It very frequently happens that the development of this tissue between the walls of adjacent epidermal cells apparently ruptures them, with the result that the fungus grows into the cells (Fig. 2). It appears to take the path of least resistance. A hypha may grow between the epidermal cells as described above and come in contact with the end wall of a palisade cell, in which case it may turn and grow around the cell. In other cases the hypha may branch, one branch growing around one side of the palisade cell while the other branch grows around the opposite side. In leaves which have undergone little disintegration, the lateral spread of the fungus is much more abundant in the mesophyll than in the palisade region. On the other hand, if the leaf has undergone considerable disintegration, the hyphae ramify the palisade more freely and, at times, appear to

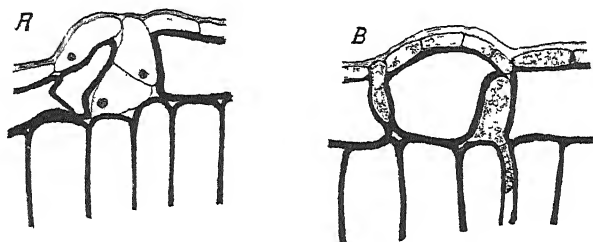


FIG. 1. A. Outgrowth from stomatal layer of *Venturia inaequalis* pushing between the walls of adjacent epidermal cells. B. Hypha from subcuticular stomatal layer pushing between palisade cells. Camera lucida drawings. ($\times 625$.)

pass through mesophyll or palisade cells. The intercellular habit of the fungus in the tissues of dead leaves which have undergone little disintegration suggests a partial explanation, at least, of the rather sparing lateral spread of the hyphae in the lamina as was noted earlier.

Perithecia may be produced either in the mesophyll or in the palisade regions of the dead leaf. Killian (10) figures the origin of a perithecium by the coiling of a single hypha about itself. Frey (4) describes the formation of perithecia in pure culture, both by the coiling of a single hypha about itself and by the coiling of two hyphae about each other. The young perithecium represented in Plate V, A of the present paper possessed a second hypha projecting from it at a point below the plane of sharpest focus, thus suggesting that it arose as the result of two hyphae coiling about each other. Evidence of both methods of origin has been found by the present writer in naturally infected dead leaves.

Killian (10) found that the portion of the hypha which is enclosed by the coiling of the end of the hypha goes to form the ascogonium. The ascogonial initial is at first more or less straight but soon takes on a curved aspect by segmentation and growth. The perithecial wall is formed from the segmentation and growth of the coiled portion of the hypha. He describes the formation of the trichogyne by the growth of one end of the ascogonium through the wall of the perithecium. The present writer finds that septation of the ascogonium may occur fairly early. Plate V, B represents a section through two perithecia. The perithecium at the right

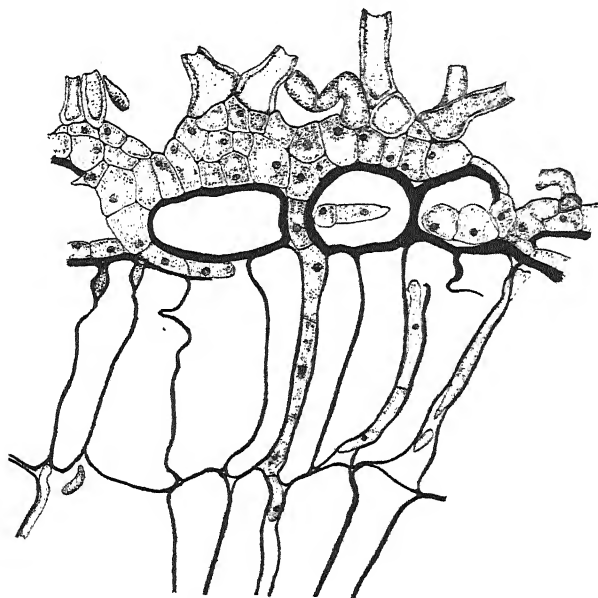


FIG. 2. Hyphae from subcuticular stromatoid layer of *Venturia inaequalis* in the palisade of the leaf. Stromatoid tissues have developed between the epidermal cells, with the results that the walls are ruptured and the fungus enters the cells. Most of the hyphae, however, are intercellular. Camera lucida drawing. ($\times 625$.)

contains the ascogonial initial before it has become curved while the one at the left contains an ascogonium which is slightly curved. Indications of a septation may be seen near one end of this ascogonium. Plate V, C represents a perithecium containing an ascogonium with four distinct septations, while D represents one containing an ascogonium with two septations, which are not discernible in the photomicrograph. No indications were found of the presence of trichogynes in any of these cases. It would seem, therefore, that septation of the ascogonium may occur before the trichogyne is formed. Plate V, E represents a perithecium in which one end of the ascogonium is

apparently pushing through the perithecial wall to form the trichogyne. A long trichogyne is shown in Plate V, F.

Frey (4) found that the ascogonium begins to lose its identity soon after an antheridium is applied to the trichogyne. He believed that it segments into many small cells from which the asci are budded. Killian (10), on the other hand, figured the remains of the ascogonium at the base of the perithecium with ascogenous hyphae arising from it. The present writer has noted the remains of this structure in perithecia which clearly showed the presence of ascogenous hyphae. Plate V, H represents a perithecium in which the ascogonium has begun to disappear, while I represents one in which only the remains of the ascogonium are visible. Plate V, J represents a somewhat more advanced perithecium with ascogenous hyphae. Killian (10) figured the ascogenous hyphae as both hooked and clubbed structures. The present writer has seen a few instances of hooked structures; but, as a rule, the ascogenous hyphae are not of any characteristic shape. Some are wedge-shaped with the narrow end in the region of the ascogonium.

At the time the ascogonium begins to disappear the lumen of the perithecium is gradually filled with hyphae. Killian (10) describes the origin of "paraphyses" from the cells of the inner wall of the perithecium. At the stage when the ascogonium takes up a position at the base of the ascocarp, the present writer has noted the presence of short projections apparently coming from any point on the inner wall of the perithecium. Later the entire lumen is filled with hyphae from these processes, which for the most part appear to arise from the inner perithecial wall above the ascogonium (Pl. V, J). If these hyphae originated from the wall of the perithecium they were not true paraphyses. They gradually disappeared from the lumen as the perithecium approached maturity (Pl. V, J, K and Pl. VII, A, B, C). In fully mature perithecia from nature they were rarely visible. If, however, the perithecia were forced to maturity in the laboratory, the hyphae might be found, although they showed signs of disintegration.

In tracing the development of perithecia, stages have been designated as follows: "simple coil" (Pl. V, A, B), extending from the time of the first appearance of perithecial initials until the ascogonium appears as a curved structure; "ascogonial" (Pl. V, B-H), from the time the ascogonium is fully differentiated until it begins to disappear; "filamentous" (Pl. V, I), from the time the ascogonium begins to disappear and the lumen of the perithecium is filled with the hyphae until the ascogenous hyphae appear; "pre-ascus" (Pl. V, J), from the time the ascogenous hyphae appear until the asci begin to appear; "early ascus" (Pl. V, K and Pl. II, A), from the appearance of the asci until the first ascospores are delimited; "delimitation" (Pl. VI, B), from delimitation of the ascospores until they become

olivaceous; "olivaceous" (Pl. VI, C), from the appearance of the first olivaceous ascospores until the first discharge; and "discharge," the first observed ejection of the ascospores. It should be noted that, in some cases, discharge may not take place from perithecia containing olivaceous ascospores even though they are kept moist for a considerable period. This is probably because the ostiole is not open at the time (see Wallace, 15).

Influence of the Time of Leaf-fall in 1924

During the autumn, winter, and spring of 1924-25 a detailed study was made of the development of perithecia in the successive collections of leaves which were taken from the tree and placed on the ground at intervals during the autumn and early winter. A brief preliminary report of this work has been made by Keitt and Wilson (9). A more detailed account follows.

Since the mode of penetration of the fungus from the subcuticular stromatoid layer into the inner tissues of the dead leaf has been described earlier, it will not be taken up here. It is necessary, however, to describe the progress of penetration in relation to the time of leaf-fall. The first fixation from living leaves on the tree, made on October 6, showed that the fungus was present in the leaves as subcuticular stromatoid layers, with no evidence that it had begun to penetrate the lamina. Fixations of apparently living green leaves from a Virginia (crab) tree on October 15 and October 20 showed that in some instances short outgrowths from the stromatoid layer had penetrated between the epidermal cells and a short distance into the palisade layer (Fig. 1). Material fixed prior to November 2, when the leaves were killed by freezing, failed to show any further progress of the fungus into the lamina of leaves which remained on the tree. After this date the fungus penetrated more extensively into the tissues of the dead leaves which remained on the tree, but fixation made as late as December failed to show any evidence of initiation of perithecia in these leaves. Fixations of leaves from the trees of the Wealthy and an unknown variety did not show the fungus in the lamina at any time previous to November 24. After this date it was found commonly in the inner tissues of the dead leaves on the trees.

The results presented in table 1 show that the time of leaf-fall in this year had a marked influence on the time the ascospores were matured the following spring. Unfortunately, owing to the writer's absence from Madison after May 11, 1925, a close study was not made of the maturation and discharge of perithecia in collections made on and after October 7, 1924. It was determined, however, that the initial discharge from these collections took place between May 25 and June 10, 1925. It is noteworthy that the perithecia in leaves of the December 17 collection were mature by June 10.

but that those of the February 9 collection failed to mature even after being placed in favorable moisture and temperature conditions on June 10 and kept thus for three weeks.

Further evidence that the perithecia in leaves which were taken from the tree early in the autumn were more advanced than those in leaves collected later is given in table 2. The time required to bring the ascospores in 100 per cent of the asci to maturity when favorable moisture and temperature were supplied in the laboratory in March varied from 10 days in the August 12 collection to 22 days in the November 10 collection. Under field conditions this difference in time would have been much greater.

TABLE 2.—*Relation of the time of leaf-fall to the maturation of ascospores of Venturia inaequalis, Madison, Wis., 1924-25*

Date of leaf-fall	Percentage of asci ^a which contained olivaceous ascospores on the stated dates of observation: ^b						
	Mar. 21	Mar. 24	Mar. 28	Apr. 2	Apr. 4	Apr. 6	Apr. 9
Aug. 12.....	30	85	100	100	-----	-----	-----
Aug. 26.....	1	65	100	100	-----	-----	-----
Sept. 9.....	0	45	70	90	100	-----	-----
Sept. 23.....	0	20	50	80	100	-----	-----
Oct. 7.....	0	0	20	66	80	100	-----
Oct. 31.....	0	0	12	22	65	80	100
Nov. 10.....	0	0	2	50	60	90	100

^a Percentage of the total number of asci observed. The more advanced perithecia were picked from the leaves for these examinations.

^b Sub-samples of leaves were taken from the stated collections on March 18 and placed in moist chambers at 16° C.

Further analysis of the data collected during the autumn of 1924 indicates that perithecia were initiated at different rates in leaves which fell at intervals during the autumn. When the average daily increase in diameter of perithecia is calculated from (1) the average diameter of the more advanced perithecia at the date of first measurement, and (2) the number of days from leaf-fall to the date of first measurement, the results of column four in table 3 are obtained. It would seem from these data that the most favorable time for initiation and early growth of perithecia during the autumn of 1924 was through the month of September and the early part of October. In 36 days perithecia were produced in the September 23 collection which were of practically the same size and stage of development as the perithecia in the August 12 collection 65 days after leaf-fall. Furthermore, there is a successive increase in the average daily growth of perithecia

in the August 26 and September 9 collections. There was a sudden diminution of average daily growth of perithecia in collections made after September 23. It was found that the perithecia were not initiated in the November 10 collection until three months after leaf-fall. A further discussion of the data in table 3 will be taken up in the section on temperature relations.

TABLE 3.—*Relation of temperature and precipitation to initiation and early development of perithecia of *Venturia inaequalis* in leaves placed on the ground at intervals during the autumn, Madison, Wis., 1924*

Date of leaf-fall	Days from leaf-fall to first measurement	Diameter of perithecia at first measurement	Average daily increase in diameter of perithecia	Average mean temperature of the 15 days following leaf-fall	Total precipitation of the 15 days following leaf-fall
	No.	Microns	Microns	° C.	Inches
Aug. 12.....	65	42	0.6	22	2.58
Aug. 26.....	64	57	0.9	18	1.61
Sept. 9.....	50	49	1.0	15	1.49
Sept. 23.....	36	39	1.1	13	0.40
Oct. 7.....	56	20	0.4	15	0.01
Oct. 31.....	81	30	0.4	7	2.23
Nov. 10.....	90	35	0.4	6	0.50

A very pertinent question arises from the consideration of the data just discussed. Are perithecia initiated and do they develop during mid-winter? From the evidence at hand it would seem that perithecia were initiated during the winter of 1924-25. Fixations of leaves taken from the tree on November 10 and a second fixation from the same collection on December 5 after a period on the ground failed to show any evidence of perithecia. On January 20, however, early stages of perithecia were found to be common in leaves of this collection. The evidence also suggests that growth of perithecia took place, at least in leaves of the later collections (October 31 and November 10). On January 20, 1925, the perithecia in leaves of the October 31, 1924, collection averaged 30 microns in diameter, while on February 12 they averaged 58 microns. On January 20 the perithecia in the leaves of the November 10 collection averaged 38 microns, while on February 12 they averaged 60 microns. The evidence does not suggest such a marked increase in size of the perithecia in the leaves of the August 12 and 26 collections. In these collections it was very infrequent that the average diameter at any one date during mid-winter was found to be greater than the average diameter of the perithecia in the preceding fixation from the same collection. This does not prove, however, that slow changes were not taking place in the perithecia of these collections. It should be borne in mind that the method of obtaining measurements was designed to ex-

clude from the record all but the earlier initiated perithecia of each collection. It is possible, therefore, that perithecia may have been initiated in leaves of these earlier collections during mid-winter.

Column two of table 4 presents data on the number of days from leaf-fall to the first observed ascospore discharge from each collection. It shows that with delayed leaf-fall there was a shortening of the time required to mature ascospores. This phenomenon will be discussed in the section dealing with temperature.

Comparing the dates of initial discharge from leaves of the various collections with the development of the host, we find that the perithecia in the leaves of the August 12 and August 26 collections began to discharge at about the time that such varieties as the Wealthy were in the "pre-pink" or "closed-cluster" stage. The September 9 and September 23 collections began to discharge during the latter part of the blooming period, while the initial discharges did not take place in the later collections until some time after the petals had fallen. It will be noted that olivaceous ascospores were present in the leaves of the August collection on April 13 but the first discharge was not noted until April 22. This was due to a spring drought which retarded the development of perithecia in all of the collections.

Influence of the Time of Leaf-fall in 1925

The results of the examination of perithecia in leaves of the Virginia (crab) variety are presented in columns four and five of table 4. The results from the McIntosh variety agree in the major details with those from the Virginia. There are, however, some significant differences, which will be discussed later.

As was found in 1924-25, the time of leaf-fall was a factor in determining ascospore maturation the following spring. In disagreement with the results of the former year, however, perithecia in leaves of the August collections did not come to maturity in advance of perithecia in leaves placed on the ground in September. The August collections had not matured ascospores by the time the last observation was made on May 25, 1926. Fixations of leaves from these collections showed the presence of perithecia, but some appeared to be abnormal. Others appeared to be normal, yet they did not mature when the leaves were placed in favorable temperature and moisture conditions on May 27 and kept thus for two weeks. This condition was met with in all of the collections made both at Madison and Sturgeon Bay during the month of August. Due to an exhaustion of the leaf material by May 25 these studies could not be pursued farther.

The first collection to mature perithecia was that made on September 16. A dry spring retarded development of the perithecia, and it was not until

TABLE 4.—*Relation of the time of leaf-fall to the time of maturity of perithecia of Venturia inaequalis, Madison, Wis.*

1924-25			1925-26		1926-27	
Date of leaf-fall	Days from leaf-fall to first observed discharge ^b	No.	Date of leaf-fall	Date of first observed discharge ^b	Days from leaf-fall to first observed discharge ^b	No.
Aug. 12.....	253	Aug. 12	None by May 25	Aug. 5	0
Aug. 26.....	243	Aug. 29	Do
Sept. 9.....	244	Sept. 5	10
Sept. 23.....	230	Sept. 16	Apr. 29	Sept. 20	71
Oct. 7 ^a	246	Oct. 19	May 15	Oct. 9	18
Oct. 31 ^a	222	Oct. 25	4
Nov. 10 ^a	212	Nov. 15	May 15	Nov. 8	0
.....	Nov. 27	May 25
Dec. 17 ^a	175	Dec. 13	None by May 25	Dec. 20	0
.....	Jan. 9	Do	Jan. 12	0

^a The initial discharges of ascospores from these collections took place between May 25 and June 10.^b Leaves were collected at frequent intervals during the spring and were tested for ascospore discharge by wetting them and placing them in moist chambers over glass slides.^c Percentage of the total number of asci observed. The more advanced perithecia were picked from the leaves for these examinations.

April 29 that the first discharge was recorded from the leaves of this collection. The trees of many of the commercial varieties of apples were in the late "closed-cluster" stage at this time. The perithecia in the October 19 and November 5 collections came to maturity at the time the apple trees were in full bloom, while the perithecia in the leaves of the November 27 collection did not begin to discharge until the petals were off of most varieties.

In conformity with the results of the previous year, the data presented in column five of table 4 show that, with delayed leaf-fall, there was a shortening of the time required to mature ascospores. The November 27 collection was placed on the ground 72 days later than the September 16 collection, yet it matured ascospores only 26 days later than that collection. This further emphasizes the ability of the fungus to produce mature perithecia in a shorter time under certain conditions than under others.

It was stated earlier that the results obtained concerning the development of the perithecia in leaves of the McIntosh variety were in general agreement with those from similar studies of the Virginia (crab). This is true in the sense that a delay in leaf-fall was correlated with a delay in the time of ascospore maturity. On the other hand, the perithecia in the McIntosh leaves were constantly less advanced than those in the parallel Virginia collections. For example, when the perithecia in the September 16 Virginia collection contained asci of approximately mature size, the corresponding McIntosh collection contained perithecia with asci just beginning to form. Further studies concerning the development of perithecia in leaves of different varieties will be discussed later.

Influence of the Time of Leaf-fall in 1926

Columns seven and eight of table 4 contain results from examinations of the Virginia leaves. The results from the leaves of the unknown variety were comparable in all major respects with those from the leaves of the Virginia. Due to the opening of the field laboratory early in the spring the writer found it necessary to leave Madison before the perithecia in all of the collections had matured. The results are sufficient, however, to show that the time of leaf-fall in the autumn of 1926 influenced the time ascospores were matured the following spring. In conformity with the results of the preceding year the collection made in mid-September was the first to mature ascospores. This suggests, as did the results of both 1924-25 and 1925-26, that September was much more favorable than August for initiation and early development of perithecia.

Ascospores were mature in the orchard at Madison in 1927 by March 14, the earliest date recorded during the three seasons that the present work

has been under way. While the September 20, 1926, collection had mature ascospores by this date, the initial discharge was not recorded until April 5. At this time the buds of all commercial varieties had begun to swell but no susceptible tissue was exposed. The perithecia in the October 9 collection began to eject ascospores at the time most of the varieties of apples were in the "green-tip" stage. The September 5 and October 25 collections contained perithecia with mature ascospores at the time the trees were in the green-tip stage, but no discharge was recorded up to the time the trees were in the closed-cluster stage. No perithecia had become erumpent on the leaves of the August 5, December 20, or January 12 collections by April 22, at the time the host was in the closed-cluster stage.

It is readily seen that comparisons between the stage of advancement of the host and the time of maturation of the perithecia do not reveal such marked differences due to delay in leaf-fall as in 1924-25 or 1925-26. This was because the host developed very slowly while perithecia developed more rapidly. If, however, the number of days between the maturation of the perithecia in the various leaf-fall collections is considered, the differences are more striking. For example the perithecia in the leaves placed on the ground on October 25, 1926, did not mature perithecia until 17 days after the first observed discharge of ascospores from the collection made September 20, 1926. It is in years such as 1927, when the host passes slowly through the period of bud unfolding, that the effects of delayed leaf-fall are minimized.

Factors which Influence the Time of Leaf-fall

Since it has been shown that early leaf-fall contributes to early maturation of perithecia, a knowledge of the factors which influence the time of leaf-fall should be of value. While a comprehensive study of this subject is beyond the scope of the present paper, the following observations have been made.

Meteorological conditions. The time of leaf-fall may be influenced by temperature and moisture in the autumn. In some instances the first freeze that is sufficient to kill the leaves may lead to retention of the foliage, while in others it may hasten defoliation. In the autumn of 1926, for example, the writer noted that trees of the Plum Cider variety lost all of their foliage within three days following the first killing freeze, while defoliation of the Northwestern Greening appeared to be checked.

Cultural practices and soil. Trees which are in good condition appear to retain their foliage longer than those in poor condition. In an effort to determine whether the application of commercial fertilizer would be practicable in delaying leaf-fall, sodium nitrate was applied to trees of the

Dudley, Wealthy, and Fameuse varieties during the growing seasons of 1925 and 1926. The results of these tests are as yet inconclusive.

Scab disease. That apple trees which are heavily infected by the scab become defoliated earlier than trees of the same variety with little leaf infection was noted by the writer in the autumn of 1926. The unsprayed Dudley trees in the experimental plots at Sturgeon Bay had lost 100 per cent of their foliage by October 27, while the sprayed trees had lost only 55 per cent.

Variety of apple. Ewert (3) reports observations on the time of leaf-fall among different varieties of apples. He states that the varieties which are most severely attacked by the scab fungus lose their leaves much earlier than those which are more resistant to the disease. He also states that the earliness or lateness of leaf-fall is a varietal characteristic. In a year when scab infection was very rare he found that the "Virginischer Rosenapfel," which is said to be very susceptible, lost its leaves much earlier than the "Antonowka," which is said to be resistant.

The present writer has made observations in the commercial apple orchards of Sturgeon Bay during two seasons when, owing to efficient spraying methods and unfavorable conditions for infection, severe leaf infection was very rare. The results, which appear in table 5, show that certain

TABLE 5.—Records of the relation of apple varieties^a to the time of defoliation, Sturgeon Bay, Wis.

Varieties	1925	1926
	Defoliation on Nov. 21	Defoliation on Oct. 27
	Per cent	Per cent
Grimes Golden	5	—
Stayman Winesap	10	—
McIntosh	15	5
Fameuse	30	10
McMahon	80	15
Northwestern Greening	90	20
Delicious	95	5
Wealthy	95	20
Virginia (crab)	98	—
Duchess	100	40
Dudley	100	55
Lubsk's Queen	100	80

^a It was not possible to make all these observations in one orchard. Consequently, other factors than varieties may have played a part in occasioning the differences noted. The same trees were observed in 1925 and 1926.

varieties consistently became defoliated before others. In a small orchard at Madison, planted to nine varieties of the same age, the McIntosh, Fameuse, and the unknown variety mentioned earlier, have consistently held their leaves later than such varieties as the Wealthy and Plum Cider, regardless of the severity of leaf infection. The unknown variety has retained many of its leaves until January. The crabs appear to have a tendency to become defoliated earlier than many other varieties, although this may be due in part to severe leaf infection.

RELATION OF VARIETY OF APPLE TO THE PRODUCTION OF THE ASCIGEROUS STAGE

A consideration of the development of the ascigerous stage in leaves of different varieties of apples is important from the standpoint of possible control methods dealing with the limitation of the primary inoculum. If it should be found that certain varieties are constant sources of inoculum while others produce little or no perfect stage, special attention could be given to the offending varieties.

It is readily apparent that there is a possibility that several factors may contribute towards making certain varieties important sources of ascospore inoculum. Of these, susceptibility to leaf infection, the disposition to early leaf-fall, and the character of the leaves themselves, as they may favor or inhibit the development of the perithecia, seem to be among the more important. It has been shown earlier that the amount of leaf infection bears an important relation to the quantitative aspects of perithecial production. It has also been shown that the time of leaf-fall has an important bearing on the time the ascospores mature in the spring. As far as the writer is aware, there has been no comparative study, hitherto, of production of perithecia in leaves of different varieties of apples. Schneiderhan and Fromme (13) report studies in which they compared the discharges of ascospores from leaves of the Rome, Stayman, and Winesap varieties throughout one season. They found no significant differences in the amount of ascospores discharged from the leaves of the three varieties. The present work on this subject has been confined to testing varieties in relation to the early and abundant development of ascospores.

Methods

Leaves were gathered from a number of varieties of apple with as comparable amounts of leaf infection as it was feasible to obtain. While the collections of each year were not all made on the same date, they were made within a few days and are closely comparable. The leaves were put in cloth bags of the type mentioned earlier and placed in the orchard for overwintering. In the spring, sub-samples were taken from each variety and placed

in moist chambers at 16° C. At intervals, 15 to 20 of the more advanced perithecia were picked from the leaves of each variety and examined. Data were gathered on the production of mature perithecia in leaves of the different varieties and on the time required for maturation of perithecia in leaves of each variety. Data were also taken on the relative abundance of the perithecia in leaves of the different varieties, but these data are not considered entirely reliable since the difference in the amount of leaf infection might have influenced the results. Table 6 contains a summary of the results obtained from these experiments.

Results

An experiment in 1924 indicated that the Charlemoff and a seedling ("Talent") were much later in maturing perithecia than the Virginia (crab), the Wealthy, or the unknown variety (mentioned earlier). Unfortunately, the available trees of the two former varieties had no leaf infection the following two seasons; and, consequently, they could not be included in the experiments of those years.

The experiments of 1925-26 and 1926-27 emphasize what seems to be a consistency of the Virginia (crab) in maturing perithecia before many of the other varieties. This was also true of the Shields (crab) in 1925-26. The Martha (crab) was somewhat later than the Virginia in both years. The results are not consistent for the Wealthy and Fameuse. In 1925-26 they both matured perithecia at the same time as the Virginia, but in 1926-27 they were considerably behind that variety. The Northwestern Greening, McMahon, and the Plum Cider were somewhat later than the Virginia in both years. It was stated earlier that the McIntosh leaves used in the leaf-fall experiments of 1925-26 were later than the Virginia (crab) in maturing the perithecia. An examination of table 6 will show that this also seemed to be true in 1926-27. All varieties under observation in the two latter years of this work produced mature perithecia in nature.

While the two years' data presented here are not sufficient to warrant any definite conclusions, they strongly suggest that perithecia are developed more rapidly in leaves of certain varieties than in others. Through experience the writer has learned to look in the leaves of the Virginia (crab), Shields (crab), and Dudley for the first mature perithecia in the spring. Besides being easier to locate on the glaucous leaves of the crabs, the perithecia are much more abundant there than on leaves of most other varieties. Although the dorsal surfaces of the young Dudley leaves are pubescent, yet it is not difficult to find the perithecia when they become erumpent. Likewise there is no great difficulty in finding the perithecia on the pubescent dorsal surfaces of the McIntosh leaves after they break through the epidermis.

TABLE 6.—*Relation of variety to the production of the perfect stage of Venturia inaequalis in apple leaves, Madison, Wis.*

Variety	Experiment of 1925-26			Experiment of 1926-27		
	Asci with mature ascospores after: ^a		Per cente	Asci with mature ascospores after: ^b		Per cente
	Three days' forcing	Eleven days' forcing		Seven days' forcing	Eleven days' forcing	
Virginia (crab)	40	100		32	60	90
Powers (crab)	0	65		50	50	80
Martha (crab)	0	20		0	29	50
Shields (crab)	28	50		—	—	—
Whitney (crab)	0	0		—	—	—
Wealthy	43	94		0	0	0
N. W. Greening	0	100		0	0	18
Patten Greening	—	—		0	0	0
Fameuse	49	75		0	0	0
McIntosh	—	—		0	0	7
McMahon	0	50		0	0	0
Dartt's Hybrid	—	—		30	67	80
Winter Banana	—	—		0	26	59
Plum Cider	0	0		0	0	0
Arkansas Black	—	—		0	0	15
Babbitt	—	—		0	12	40
Windsor	—	—		0	0	0

^a Sub-samples of collections taken from the tree on October 10, 1925, and overwintered on the ground, were brought into the laboratory on April 11, 1926, and subjected to favorable temperature and moisture conditions for maturation of perithecia.

^b Sub-samples of collections taken from the tree on October 5-11, 1926, and overwintered on the ground, were brought into laboratory on February 23, 1927, and subjected to favorable temperature and moisture conditions for maturation of perithecia.

^c Percentage of the total number of asci observed which contained olivaceous ascospores. The more advanced perithecia were picked from the leaves for these examinations.

RELATION OF TEMPERATURE TO THE DEVELOPMENT OF THE ASCIGEROUS
STAGE

It was evident from a consideration of the influence of the time of leaf-fall on the development of the perfect stage that this was not the only factor which was concerned in inducing variability in the time of maturity of ascocarps. If the time element alone were concerned, one would expect that leaves placed on the ground two months apart would mature ascospores two months apart. That this was not the case is clearly shown in table 1. Furthermore, leaves placed on the ground at different times during the autumn of 1924 did not initiate perithecia at the same rate, as is shown in table 3. It was with these facts in mind that the influence of temperature on the development of perithecia was studied.

The literature on temperature in relation to the development of the ascigerous stage of *V. inequalis* is very fragmentary and inconclusive. In his work on the *Venturia* on *Pyrus coronaria*, Jones (7) made some observations on the production of perithecia in artificial media. He found that they were produced at 10°, 16° and 26° C. Howitt and Evans (6), through observational data, sought to correlate the development and the time of initial discharge of ascospores in the spring with temperature and precipitation. They conclude that, during the six seasons they were concerned with, the average mean temperature of January, February, and March seemed to bear a more direct relationship to the development and time of discharge of the ascospores than did the amount of precipitation from April 1 to the date of first discharge. They further report that the average mean temperature of January, February, and March was more closely related to the development of perithecia and the time of discharge than the mean temperature of the days of April and May preceding primary discharge. Under controlled temperature conditions, Keitt and Jones (8) found that ascospores matured slowly in leaves kept at 4° C. and at 7° C., but there was a successive increase in the rate of maturation at 12° and 16° C., with an optimum near 20° C. At 24° C. they found that maturation was definitely checked. They also found that discharge of ascospores occurred at temperatures ranging from 0.5 to 32° C.

Methods

The present work has been carried out with the aim of subordinating all factors other than temperature. The results have been obtained by subjecting the fungus to controlled temperatures while growing in pure culture and in naturally infected dead leaves.

The fungus has been grown on oatmeal, apple-leaf, malt, apple-peel, synthetic, potato dextrose, and cornmeal agars. Preference has been given to the oatmeal and apple-leaf agars, for reasons which will be discussed

later. The oatmeal agar was made from 50 gms. of rolled oats and 15 gms. of agar a liter. The apple-leaf agar was made by steaming 100 gms. of dead apple leaves in 1,000 cc. of water for 30 minutes, passing the decoction through filter paper and adding 15 gms. of agar. The methods have been standardized as far as possible. Petri dishes of uniform size were poured with 15 cc. each of the desired medium. After the agar had solidified the plates were treated with 0.5 cc. each of a conidial suspension from a 10- to 12-day-old culture of the organism. The suspension was allowed to spread over the agar as evenly as possible and the cultures were incubated at room temperature. When the fungus had become macroscopically visible on the agar, and before perithecia had begun to form, the petri dishes were placed in triplicate at the desired temperatures. Control was effected within 1° C. To avoid excessive desiccation at the higher temperatures, the petri dishes were placed in large glass chambers with moist absorbent cotton. After the cultures had been kept at the controlled temperatures for from 5 to 10 days, depending on the medium used, data were taken on the abundance and the diameters of the perithecia in each plate. The number of perithecia was counted in 100 fields of the microscope (a microscopic field was 2.4 square millimeters in area). The observations were made in areas in which the fungus occurred in abundance. Measurements were made of 50 perithecia in each plate when that number could be found.

In the studies of the effect of temperature on initiation and early development of perithecia in leaves, the leaves were taken from the tree before the fungus had grown below the stromatoid layer. Several of these leaves were cut into pieces and one piece from each was placed in a moist condition at each of the desired temperatures. After 10 to 16 days the leaves were removed and cleared by the chloral hydrate-potassium hydroxide method mentioned earlier (Peace, 12). Measurements were then made of 100 perithecia in the leaves kept at each temperature.

The leaves which were used for the effect of temperature on the growth of asci and delimitation and maturation of ascospores were from a supply which had been taken from the tree on a certain day in the autumn and placed in the orchard until needed. When the asci began to appear, a number of leaves were selected which bore the diffuse type of lesions scattered uniformly over the surfaces. These leaves were cut into pieces and one piece from each placed in a petri dish and kept at the desired temperature. The moisture was controlled, as nearly as possible, by a standard method of wetting. Examinations of the perithecia were made at intervals by picking a number of the more advanced ones from each piece of leaf and crushing them in water on a glass slide. Records were made of the percentage of asci which were three-fourths mature size or larger (including those with delimited and mature ascospores), the percentage with delimited ascospores

(including those with mature ascospores), and the percentage with mature ascospores. In these results and those shown in the other tables and graphs, the writer wishes to interpret the quantitative aspect very conservatively. While an earnest attempt has been made to apply quantitative methods as fully as feasible in the collection and presentation of data, the nature of the problems is such that the results are best considered as semi-quantitative. The purpose, in the main, has been to show trends and relationships with accuracy adequate for the purposes served, rather than to seek strictly quantitative data on matters too complex to be treated in a rigidly quantitative way in the present state of our technique and resources.

Growth of the Fungus into the Interior of the Dead Leaf

Experiments have shown that the hyphae of *V. inaequalis* will grow from the subcuticular stromatoid layer into the lamina of the leaf within three days at temperatures ranging from 4° to 28° C., but no growth was found in the leaves which had been kept in a moist condition for one week at 31° C. Fixations of leaves which had been kept at -4° C. for 20 days showed an occasional hypha in the interior. An examination just before this experiment was begun showed no hyphae in the interior of the leaves. To avoid mistaking the hyphae of other fungi for those of *Venturia*, no hypha was considered to be that of *Venturia* unless it was seen to come directly from the stromatoid layer. The characteristic conidiophores identified the stromatoid layer of *Venturia*.

There was a distinct difference in the character of the hyphae in the leaves kept at the different temperatures. Whereas, the hyphae in the leaves which were kept at 4°, 10°, and 16° C. were comparatively thin-walled and of light color, those in the leaves which were kept at the higher temperatures (21°, 24°, and 28° C.) were dark olivaceous to brown in color and moniliform in shape. On certain solid media similar effects of temperature were noted. At 28° C. the hyphae were very slender and often grew in a sinuous fashion through the substratum.

Initiation and Early Growth of Perithecia

The growth of the fungus in culture is only approximately similar to its growth in the leaf after leaf-fall. Most media which are favorable for vegetative growth of the fungus are also favorable for production of conidia. The abundant production of conidia undoubtedly has a tendency to delay initiation of perithecia. Furthermore, the fungus responds differently to temperature when growing in different media. On malt agar, for example, an increase of temperature to 24° C. was found to be accompanied by an increase in the production of mycelium and of sterile aerial hyphae. When

the fungus was exposed to temperatures ranging from 4° to 28° C. it was found that the greater number of conidia was produced at 10°, 13°, and 16° C., with an apparent sharp diminution in production at 19° C. This example is presented for the reason that in two experiments on this agar the perithecia were produced in greater numbers at 19° C. Had malt agar alone been used, the optimal temperature for initiation of perithecia would have appeared to be near 19° C. When the fungus was grown on agar made from dead apple leaves, on the other hand, temperature did not appear to have so marked an effect on vegetative development as was the case when it was grown on malt agar. It is noteworthy that comparatively few conidia were produced on this agar and their abundance was not affected to a noticeable extent by the range of temperature used. Most of the experiments on the relation of temperature to initiation and growth of perithecia were carried out on this medium.

The growth of the fungus on oatmeal agar more closely paralleled that on agar made from dead apple leaves than did the growth on malt agar. While conidia were produced on oatmeal agar, their abundance did not vary markedly at temperatures between 7° and 21° C. Some of the experiments on the effect of temperature on the initiation and early growth of perithecia were carried out on this agar for comparison with the results from apple-leaf agar.

Figure 3 is a graphic representation of results obtained when the fungus was grown in apple-leaf agar, oatmeal agar, and in naturally infected dead leaves. With the exception of the results from malt agar, all experiments indicate that the optimal temperature for initiation and early growth of perithecia lies between 13° and 16° C. In both leaves and apple-leaf agar the optimum appears to be nearer 13° C. In oatmeal agar, on the other hand, some of the experiments indicated that the perithecia were produced in greater abundance and were of larger size at 16° C., while in others they were more numerous at 13° C. All the data presented in figure 3 agree in one respect; *viz.*, that there was a sharp diminution of initiation and early growth of perithecia at 19° C.

The data in table 3 indicate that perithecial initiation and early growth proceeded more rapidly in leaves which were placed on the ground in September than in those which were similarly placed either before or after September. The meteorological data presented in figure 4 shows that there was a gradual decrease of the mean temperature as autumn progressed. When the mean temperature of the 15 days following each of the leaf-fall collections is calculated and the average daily increase in diameter of perithecia is compared, as in columns four and five of table 3, it is seen that the mean temperatures following the September 9 and September 23 collections are distinctly within the optimal range found for initiation and

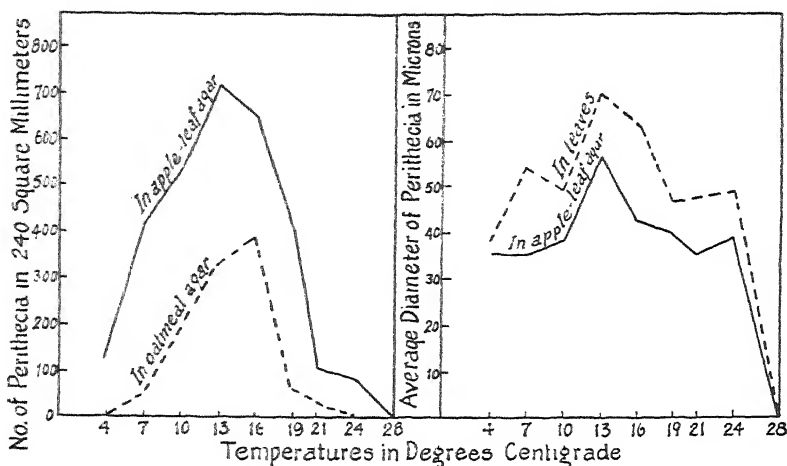


FIG. 3. Relation of temperature to the initiation and early development of perithecia of *Venturia inaequalis* in pure culture and in naturally infected apple leaves.

early growth of perithecia in culture and in leaves. The low daily increase in diameter of perithecia recorded for the October 7 collection cannot be attributed to the temperature immediately following the collection of these leaves, since it is within the optimal range. The rainfall, on the other hand, probably accounts for the difference. Whereas the September 23 collection was followed within 15 days by 0.40 inch of precipitation in four rains, there was only 0.01 inch of precipitation within 24 days following the October 7 collection. The low temperatures prevailing after the October 31 and November 10 collections were placed on the ground probably account for the low daily increase recorded for these collections.

Additional data presented in table 7 show what seems to be a correlation between the temperature following leaf-fall and the initiation and early development of perithecia. It will be seen that in both 1925-26 and 1926-27 the most advanced perithecia were found, when examinations were made in the spring, in leaves which were placed on the ground in September. In both of these years the mean temperatures for the 15 days following these collections were nearer the optimal temperature found for the initiation of perithecia under controlled conditions than were the mean temperatures for similar periods following the August collections. Both high temperatures and lack of moisture (Fig. 4) may have retarded the perithecia in the August collections of 1925 and 1926. On the other hand, the rainfall for the 15 days following the September 5, 1926, collection, although not quite as abundant, was more evenly distributed than the rainfall for a similar period following the September 20, 1926, collection, yet the peri-

thecia in the September 20 collection were distinctly more advanced than those in the September 5 collection. It appears, therefore, that in this case temperature was the determining factor.

Two experiments were conducted during the autumn of 1926 in which leaves were taken from the tree and samples were placed in a moist condition at 4°, 10°, 16°, and 24° C., respectively. After 15 days in one experiment and 20 days in the other the leaves were removed from the respective temperatures and placed in the orchard. In the spring the samples were taken from the orchard and placed in moist chambers at 16° C. In the first experiment, perithecia in the leaves which had been kept for 15 days at 10° and 16° C. came to maturity only slightly ahead of the perithecia in those which had been kept at 4° and 24° C. In the second experiment, however, in which the leaves had been kept at the several temperatures for 20 days, the perithecia in those kept at 10° and 16° C. were distinctly more advanced than those in leaves kept at either 4° or 24° C.

In order not to overestimate the direct effects of temperature on perithecial development in naturally infected dead leaves, it should be borne in mind that high temperatures are favorable to development of various saprophytic organisms in the leaves, provided the moisture conditions are favorable. The leaves which were placed on the ground in August were almost always in more advanced stages of disintegration in spring than those which were placed on the ground later in the season. The rapid disintegration of the leaves might possibly have influenced the development of *Venturia* in those leaves. The direct effect of temperature is, however, of importance. High temperatures seemed to have a tendency to induce aberrant development of the perithecia, especially if abundant moisture was present. In pure culture, the perithecia produced at temperatures of 21° and 24° C. were more irregular in shape than those produced at 13° or 16° C.

The evidence given earlier suggests that initiation and early growth of perithecia occurred between December 5 and January 20, 1924-25. A study of the thermograph records which were taken between these two dates at the place where the leaves were overwintered showed that the temperatures at the surface of the ground beneath the snow remained constantly below 0° C. Similar records made during the winter of 1926-27 show that during December, January, and February the temperature at the surface of the ground beneath the snow seldom fell below -5° C. During the middle of the day the temperature often rose to -1° or -2° C. When the ground was bare, however, considerably lower temperatures were recorded. Under these conditions, of course, the temperature fluctuated much more than when the ground was covered by snow. During the middle of some of the warmer days, temperatures above 0° C. occurred. To determine

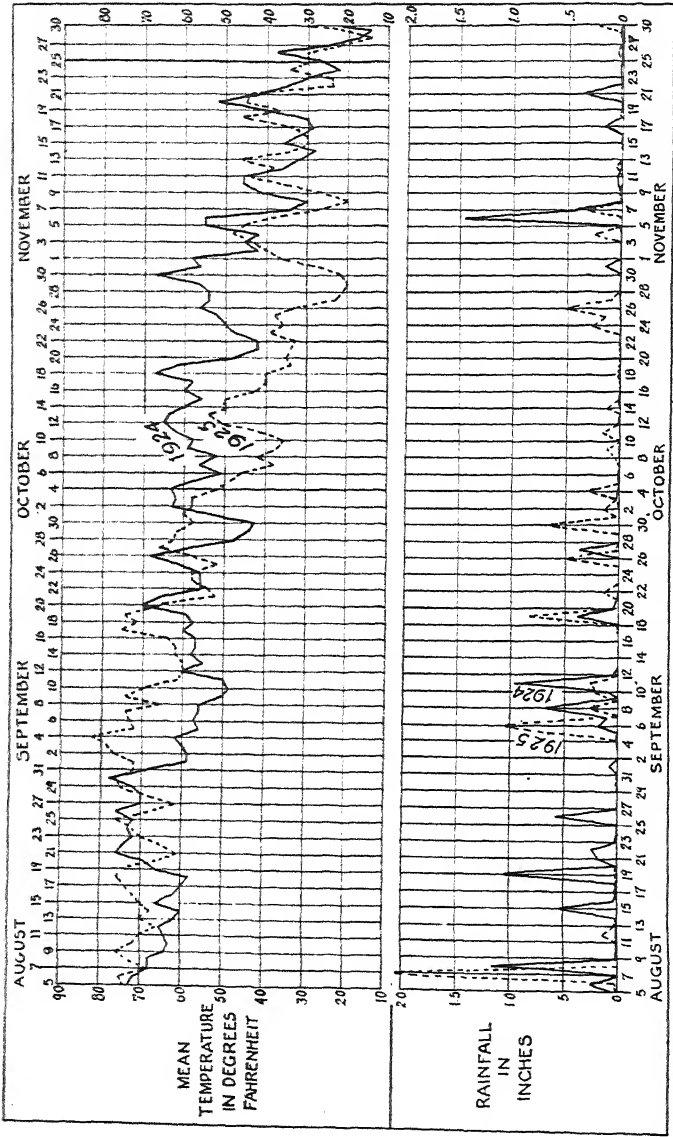


FIG. 4. A record of the temperature and rainfall at Madison, Wisconsin, during the autumns of 1924 and 1925.

TABLE 7.—*Relation of the temperature following leaf-fall to the development of perithecia of Venturia inaequalis, Madison, Wis.*

1925-26			1926-27		
Date of leaf-fall	Mean temp. of the 15 days following leaf-fall °C.	Stages of development of perithecia on April 23, 1926	Date of leaf-fall	Mean temp. of the 15 days following leaf-fall °C.	Stages of development of perithecia on March 7, 1927
Aug. 12	22	None erumpent	Aug. 5	21	None erumpent
Aug. 29	23	do	Sept. 5	17	Early ascus
Sept. 16	17	Delimitation	Sept. 20	15	Delimitation
Oct. 19	0	Early ascus	Oct. 9	9	Early ascus
Nov. 5	2	None erumpent	Oct. 25	6	do

^a Stages of development: None erumpent = no perithecia erumpent on the surface of the leaves; Early ascus = asci just beginning to appear; Delimitation = ascospores visible but not yet olivaceous.

whether perithecia would be initiated and develop at temperatures which were kept constantly below $0^{\circ}\text{C}.$, leaves were collected from the tree in the autumn of 1926 before they had been killed by frosts. Free-hand sections of these leaves showed that the fungus had not penetrated beyond the subcuticular position. The leaves were moistened and placed in an ice-mold of a refrigerating machine. The temperatures in this mold averaged $-4^{\circ}\text{C}.$ and never went above $0^{\circ}\text{C}.$ In January, during repair of this machine, workmen removed the leaves from the ice-mold. It was not until about eight days later that the writer discovered that they had been removed. They were by this time air-dry. Some tests showed that leaves left under the conditions that these leaves were subjected to during the eight days outside the ice-mold would become air-dry within three or four days. Upon making free-hand sections of the leaves which had been in the ice-mold, it was found that the fungus had penetrated to the interior abundantly and that perithecia at the early stages of fertilization were not uncommon. Unless it can be assumed that the fungus is able to grow into the interior of the leaf and initiate perithecia within five or six days, it must be concluded that at least some part of this development occurred at temperatures below $0^{\circ}\text{C}.$ The experience of the writer has been that, under the most favorable temperature and moisture conditions, comparatively abundant ramification of the hyphae in the lamina of the leaf takes place only after three or four days. The evidence, reported under the preceding heading, that a few hyphae were found in leaves which had been kept for 20 days at $-4^{\circ}\text{C}.$, lends weight to the probability that growth of the fungus occurred in the ice-mold. Further experiments, however, are necessary to establish this point definitely.

Some experiments indicated that, on certain media, initiation of perithecia was stimulated when the cultures were exposed for a short period to temperatures near $0^{\circ}\text{C}.$ Petri-dish cultures of *V. inaequalis* on oatmeal and apple-peel agars were prepared as described earlier. When the fungus became macroscopically visible, two sets of three plates each were placed at -4° , 4° , and $21^{\circ}\text{C}.$, respectively, and one set was placed at $12^{\circ}\text{C}.$ After three days one set from each of the -4° , 4° , and $21^{\circ}\text{C}.$ temperatures was transferred to a temperature of $12^{\circ}\text{C}.$ Examinations of the cultures were made at intervals and the time of appearance and abundance of perithecia were noted. It was found that perithecia appeared in a shorter time and in greater abundance in the plates which had been kept for three days at -4° and $4^{\circ}\text{C}.$ than in similar plates which had been kept constantly at -4° , 4° , 12° , or $21^{\circ}\text{C}.$, or in the plates which had been kept for three days at $21^{\circ}\text{C}.$ In one experiment two plates were placed out-of-doors for 15 hours during a night when the temperature reached a minimum of $-18^{\circ}\text{C}.$

Upon subsequent removal to a temperature of 15° C., perithecia appeared in greater abundance than in similar plates which had been kept constantly at 15° C. When these experiments were repeated with the fungus growing on agar made from dead apple leaves, no such indications were found of a stimulatory effect of short exposures to low temperatures. On the contrary, the short exposures retarded initiation of perithecia.

Growth of Asci and Delimitation and Maturation of Ascospores

Figure 5 is a graphic representation of the results obtained from one of the experiments on the influence of temperature on the maturation of ascospores in apple leaves. It shows that there were successive increases in the rate of maturation at 4°, 7°, 10°, 13°, 16° and 19° C. The rate of maturation was almost maintained at 21° C., but at 24° C. there was a distinct decrease. At the latter temperature the contents of the asci had undergone disintegration. In some instances the ascospores had been delimited but apparently no wall had been formed around them, with the result that the apical spore often effectively plugged the ascus. Attempts at forcing discharge from such perithecia met with but little success. Additional experiments in which leaves were kept at the higher temperatures for eight days showed that temperatures near 24° C. and above were deleterious to the normal maturation of ascospores if the leaves were in a moist condition, but no marked effect was noted within a similar period when the leaves were kept air-dry while exposed to the high temperatures.

It is noteworthy that the curve represented in figure 5 corresponds very closely to that obtained by Keitt and Jones (8) for germination of ascospores in water. It also conforms, to a marked degree, with their results on the temperature relations of the fungus during infection and incubation in that the optimum in all of these cases appears to be near 20° C.

It was noted earlier that in 1924-25 and 1925-26 with a delay in leaf-fall there was a shortening in the time between leaf-fall and the first discharge of ascospores (Table 4). There were 253 days between the time the collection was placed on the ground on August 12, 1924, and the time the first discharge was observed from this collection; whereas only 175 days elapsed between the time the December 17, 1924, collection was placed on the ground and the time the first discharge was observed. The magnitude of the difference and the consistency of the trend of the data warrant the conclusion that some factor other than time of leaf-fall played a rôle in determining the time necessary for the maturation of ascocarps. Since the data in table 4 show a consistent trend in one direction, it would be expected that the factor involved would likewise show a consistent trend. Since the amount and distribution of rainfall fluctuated greatly, it could

hardly be expected that it might be concerned in inducing the variability mentioned above. Temperature, on the other hand, had a constant trend downward during the autumn months and a constant trend upward during the spring. The explanation of this phenomenon was sought, therefore, through a study of the influence of temperature on development of perithecia at stages preceding maturation. It will be recalled that the optimal temperature for initiation and early growth of perithecia appeared to be near $13^{\circ}\text{C}.$, while the optimum for maturation of ascospores was near $20^{\circ}\text{C}.$ This means that there was a shift from a lower to a higher range of thermal requirements. This shift could take place in either of two possible ways: (1) a sudden change from the lower to the higher requirement, or (2) a gradual shift. What are the effects of the transition from a lower to a higher optimal thermal requirement during a time when the temperatures are gradually declining, and what is the result when the temperatures are gradually rising during the spring? An attempt was made to answer these questions through a study of the effect of temperatures between 4° and $16^{\circ}\text{C}.$ on the growth of asci and delimitation and maturation of ascospores. Leaves bearing perithecia in the early stages of ascus formation were cut into pieces and one piece from each leaf was kept in a moist condition at 4° , 7° , 10° , 13° , and $16^{\circ}\text{C}.$, respectively. At frequent intervals several of the more advanced perithecia were picked from the leaf fragments at each temperature. The perithecia were crushed in water on

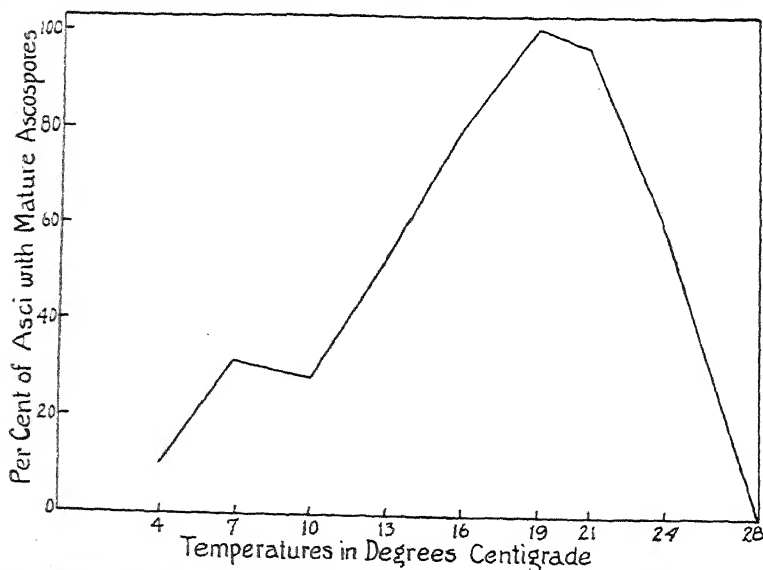


FIG. 5. Relation of temperature to the maturation of ascospores of *Venturia inaequalis* in naturally infected apple leaves.

a glass slide and counts were made of the total number of asci in each perithecium. Records were then made of: (1) the number of visible asci which were three-fourths mature size or larger, including those which contained delimited ascospores and those which contained mature ascospores; (2) the number of asci which contained delimited ascospores, including those which contained mature ascospores; and (3) the number of asci which contained mature ascospores. On the basis of the total number of asci observed, the percentage of asci which were three-fourths mature size or larger, the percentage of asci with delimited ascospores, and the percentage of asci with mature ascospores were calculated. Figure 6 is a graphic representation of the averages of the results of three observations from the same experiment.

The results of three experiments agree in all major aspects. Whereas the curve representing ascus growth rises rapidly with additional increments of temperature above 4° C., the curve representing maturation of asci remains low at 4°, 7°, and 10° C., but rises rapidly at 13° and 16° C. In addition to this, it was found that at 4° C. 85 per cent of the asci became three-fourths mature size or larger in eight days, while no mature ascospores were produced in that time. At 7° C. 98 per cent of the asci became three-fourths mature size or larger in eight days, while only 2 per cent of the asci matured ascospores. In the three experiments the curve representing delimitation of ascospores also showed a lag at the two lowest temperatures at the time the first observations were made. The average of three observations from one experiment, however, gave a curve which was intermediate between the curve representing maturation and that representing ascus growth (Fig. 6). This suggests that the temperature requirements for delimitation were intermediate between those for ascus growth and maturation of ascospores; but, since the other two experiments were not carried farther than the first observation, conclusive evidence on this point is lacking. In any case, the results of the three experiments are in agreement in that growth of asci takes place more readily at low temperatures than does maturation of ascospores. The writer considers this to be evidence of a gradual change in thermal requirements as the asci approach maturity.

If, as indicated by the results presented in figures 3, 5, and 6, the optimal range of temperature for initiation and early growth of perithecia is distinctly lower than that for maturation of ascospores, and the transition from the lower to the higher range takes place gradually as the perithecia develop, a possible explanation is afforded of how temperature may have been concerned in inducing the variability which was found in the time necessary for development of the perithecia in the leaf-fall collections of both 1924-25 and 1925-26 (Table 4). The perithecia in the leaves which

had been placed on the ground in early autumn (September), having reached an advanced stage, were checked in their development by the gradually declining temperatures of late autumn. The perithecia in the leaves which had been placed on the ground later in the autumn, on the other hand, being less advanced, and having a lower thermal requirement, were able to develop at a greater rate for a longer time in the late autumn than the more advanced perithecia. Not only this, but the less advanced perithecia would respond more quickly to the gradually rising temperature of spring. The evidence which was reported earlier suggests a further possible factor which may have contributed to this variability; *viz.*, growth of the less advanced perithecia during midwinter. This possibility, however, lacks conclusive proof.

Under Wisconsin conditions, the fact that the higher thermal requirement has not been entirely reached by the time the asci appear is a distinct advantage to the fungus. The occurrence of temperatures a few degrees above 0° C. during late winter or early spring is accompanied by development of the ascogenous elements. This places the asci in such condition that only a few days of temperature above 10° C., with sufficient moisture, are necessary to permit maturation of ascospores. It can be seen how a

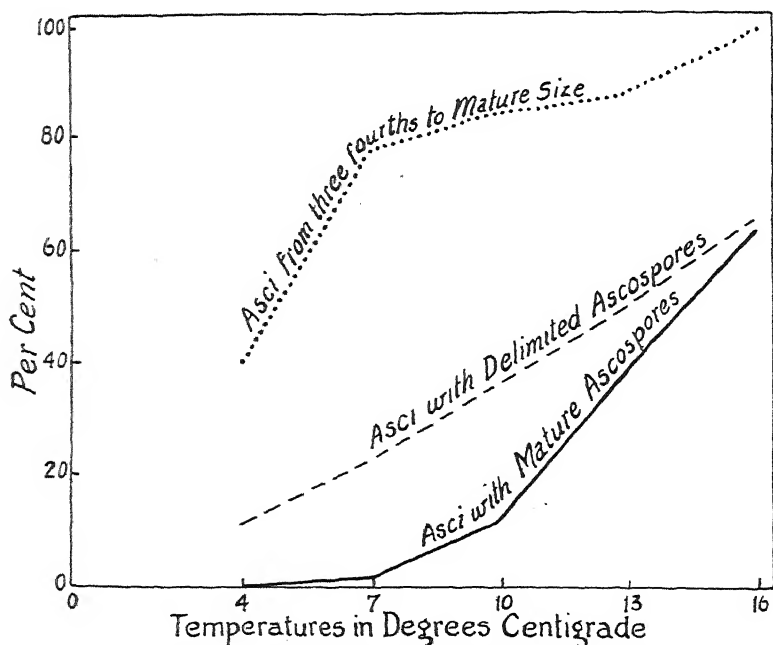


FIG. 6. The relation of temperature to ascus growth and the delimitation and maturation of ascospores of *Venturia inaequalis* in naturally infected apple leaves.

relatively short period of favorable temperature during the spring might lead to more development than would occur over a longer period during which the temperature was slightly below the critical range. An example of the rapid development of perithecia during a short period of favorable temperatures was witnessed by the writer at Madison in the spring of 1927. By the end of February the most advanced perithecia in nature were at about the "pre-ascus" stage. The first half of March was marked by temperatures above the normal for that month. The snow rapidly disappeared, leaving the leaves in a moist condition for several days. Thermograph records show that between March 7 and 21 there was a total of 120 hours when the temperature taken among the leaves remained above 4° C. Temperatures above 10° C. were recorded for 42 hours during this period. Frequent examinations of perithecia in leaves which had been placed on the ground on September 20, 1926, showed that asci developed rapidly during this period. By March 14 about 2 per cent of the asci in the more advanced perithecia contained mature ascospores. Following March 21 there was a period when the temperatures among the leaves seldom went above 4° C. No discernible maturation of ascospores took place during this period, but practically one hundred per cent of the asci in the more advanced perithecia had reached approximately mature size by March 26.

RELATION OF MOISTURE TO THE DEVELOPMENT OF THE ASCIGEROUS STAGE

Several authors have contributed to our knowledge of the relation of moisture to the discharge of ascospores of *V. inaequalis*. Frey and Keitt (5) observed that discharge in the orchard occurred when the leaves were wet by rain. Wetting by dew was not found to give discharges of consequence. As was pointed out earlier in this paper, Howitt and Evans (6) report a closer relation of the average mean temperature of January, February, and March to the time of primary ascospore discharge than the amount of precipitation from April 1 to the time of primary discharge. Owing to the nature of their data they were not able closely to define the relationship of moisture to ascospore development. The importance of rain in the spring in hastening ascospore maturity has been pointed out by Keitt and Jones (8), who observed that drought retarded development and primary discharge. Under controlled conditions they state that . . . "discontinuous wetting led to more rapid maturation than continuous wetting," and . . . "at 16° C. and a relative humidity of 78 per cent . . . the maturation of perithecia was checked until the leaves were thoroughly wet."

The above-mentioned studies were concerned chiefly with the effects of moisture on the later stages of ascocarp development. So far as the pres-

ent writer is aware, no attempt has been made to determine the relation of this factor to the initiation and early development of perithecia. A knowledge of this sort would be instructive, inasmuch as the amount of precipitation during the autumn might possibly influence the time of ascospore maturity the following spring. Experiments have therefore been conducted in an effort to determine the relation of moisture to the entire process of perithecial production.

Methods

It has been difficult to devise methods whereby the moisture content of the leaves could be controlled within a narrow range. As a consequence, much of the work has been of a qualitative, rather than a quantitative, nature. Some experiments, however, have been conducted in which an attempt has been made to use leaves with various known moisture contents. Known weights of air-dry leaves were immersed in water for varying lengths of time. At the desired time the leaves were removed from the water, the water was removed from the surfaces of the leaves by placing them between filter papers, and a second weighing was made. The water content was calculated on the oven-dry basis.

Where no attempt was made to determine the moisture content of the leaves, the degree of moisture was regulated as follows: (1) air-dry; (2) moist enough to be fully pliable; and (3) wet. When initiation and early growth of perithecia were studied, leaves with the lesions scattered uniformly over the lower surfaces were taken from the tree and cut into pieces, and one piece from each leaf was placed in the desired moisture condition for from 10 to 12 days. The leaves were then cleared by the Peace (12) method, and the abundance and diameter of the perithecia were determined as described for the studies of the relation of temperature.

For the study of the effect of moisture on maturation of ascospores, leaves were taken from a supply in the orchard and cut into pieces. One piece from each leaf was placed in each of the moisture conditions mentioned above. At intervals the more advanced perithecia were picked from each fragment at each of the several moisture conditions and examined. The results were recorded as the percentage of the total number of asci observed which contained delimited ascospores (including those which contained mature ascospores), and the percentage containing mature ascospores.

Absorption and Retention of Water by Dead Apple Leaves

Before beginning the discussion of the results of the experiments with the fungus it seems desirable to consider the wetting properties of leaves.

It would be expected that there would be wide variations in the wetting properties of leaves, depending on their structure and the amount of disintegration they had undergone. The more or less constant type of leaves produced by each variety of apple would lead one to expect, however, that the wetting property of any one variety would fall within a certain range, providing the leaves had undergone the same amount of weathering.

It was noted during the progress of the work that the leaves of certain of the crabs, upon being immersed in water, would become pliable more quickly than those of the McIntosh variety. As was earlier noted, perithecia appear to mature earlier in the Virginia (crab) leaves than in the leaves of the McIntosh. It was thought that this difference might possibly be explained, in part, by the rate of water absorption and water retentivity possessed by the leaves. It might be expected that during autumns and springs of little rainfall the perithecia would develop most rapidly in those leaves which had the ability quickly to absorb and hold the moisture of each light rain, or possibly of dew. It could hardly be expected, on the other hand, that such leaf properties would be of any great value to the fungus during seasons of abundant rainfall, since even the leaves which were the most resistant to wetting would become practically saturated during prolonged moist periods. To obtain evidence on this question, leaves of the Virginia (crab) and McIntosh varieties which had been placed on the ground at the same time in the autumn were brought into the laboratory. Known weights of these leaves were placed, with noted surface uppermost, on screens, and subjected for 10 minutes to a uniform spray of water. The water was then removed from their surfaces by the method described earlier, and a second weighing was made. Averages of 14 such experiments showed that the crab leaves took up 57 per cent of their oven-dry weight in water, while the McIntosh leaves took up 42 per cent. Experiments to determine the rate of water loss from the leaves of these two varieties failed to show any constant differences. Experiments to determine whether there was any difference in the rate of growth of perithecia when the leaves of the two varieties were subjected to parallel wetting treatments are as yet incomplete and inconclusive. This phase of the investigation proved to be one which required much more work for conclusive results than was feasible in the time available.

Additional experiments indicate that leaves absorb increasing amounts of water until a point is reached where the methods used revealed no more absorption after an hour of immersion under normal air pressure. If the air of these leaves was then exhausted, cases were noted where 32 per cent more moisture was absorbed. On the other hand, leaves which had been wet in the orchard for several days were found to weigh the same after the air was exhausted as before.

Growth of the Fungus into the Interior of the Dead Leaf

Growth of the hyphae from the subcuticular stromatoid layer into the interior of leaves taken from the tree in the autumn did not take place when the leaves were kept air-dry in the laboratory for 20 days. When similar leaves were moistened, however, ramification into the lamina took place within three days.

That the moisture present in the green leaf was sufficient to permit penetration of the fungus was shown by placing such leaves in a petri-dish to prevent drying. Within three days the fungus was found in the interior of the leaf. In no case, however, was the fungus found to have penetrated the interior tissues of the leaf while it remained green, but it did so shortly after the leaf turned brown. It was noted that temperature was important in hastening browning of the leaves, those at the higher temperatures turning brown first.

Initiation and Early Growth of Perithecia

No initiation of perithecia was found in leaves which had been kept air-dry in the laboratory for one month. When leaves were moist enough to be fully pliable, however, perithecia were initiated readily. Moisture in excess of this did not seem materially to hasten initiation and early growth. Leaves were taken directly from the tree and divided into two lots. One lot was moist enough to be fully pliable, and the second was kept wet. After being kept for 12 days at 14° C., samples from each lot were cleared by the Peace (12) method. Measurements of 100 perithecia taken at random in each lot gave average diameters of 48 μ and 50 μ , respectively.

The data presented in table 3 indicate that drought retarded development of the perithecia in leaves which fell on October 7, 1924. On the other hand, although the rainfall following the September 23 collection was much less than that following the September 9 collection, yet the average daily increase in diameter of perithecia was practically the same in both cases. An examination of figure 4 shows that the rainfall following the September 9 collection was evenly distributed over the 15-day period. It is impossible to say whether dew might not have influenced the growth of the perithecia in the September 23 collection. No data have been obtained on this point, but the control experiments indicate that leaves may absorb moisture from the highly humid atmosphere of the Altmann apparatus, especially at the lower temperatures, and that perithecia may be initiated under these conditions in the absence of applications of liquid water. It can be conceived that the dews of certain types of autumn weather may be sufficient to permit initiation of perithecia.

Growth of Asci and Maturation of Ascospores

During dry periods of the spring months the perithecia seem to develop little if any. Rains followed by cloudy weather are conducive to rapid maturation of ascospores, providing the temperature is favorable. The conditions prevailing during an average spring would cause alternate wetting and drying of the leaves at intervals. In unusually wet springs, such as 1924, the leaves would remain wet for considerable periods. The question of whether protracted wet periods are favorable for maturation of ascospores is important, since early infections of the host are dependent upon comparatively long moist periods, and since, if ascospores were continually coming to maturity under these conditions, severe infections would result.

The experiment of discontinuous wetting reported by Keitt and Jones (8) was repeated with a slight modification. Leaves bearing the fungus in abundance were cut into pieces, and one piece from each leaf was placed in a petri-dish under each of the following moisture conditions: (1) wet for several minutes then placed in the dish, (2) wet for the same length of time as the first but the surface moisture removed between filter-papers before placing in the dish, (3) started wet (as No. 1) but allowed to become air-dry after two days, and thenceforth subjected to two-day periods of alternate wetting and drying. The results are presented in table 8. They are in general agreement with the results of Keitt and Jones in that the alternate wetting and drying led to more rapid maturation than continuous wetting. On the other hand, the leaves which were kept constantly "moist" contained perithecia which were at practically the same stage of development as those which had been alternately wet and dry. This does not necessarily mean that continuous moisture over a prolonged period is so favorable to normal development as discontinuous wetting. It has been found that, even at temperatures as low as 10° C., prolonged moistening of the leaves leads to aberrant development of the asci. Fixed and stained material from such leaves shows that there is often a development of vegetative tissue within the lumen of the perithecium, with the result that the asci are crowded to the top of the ascocarp. It would appear that the alternate wetting and drying as it occurs in nature leads to more normal development of the perithecia.

Experiments in which attempts were made to hold the moisture contents of the leaves at a certain known point have not been very satisfactory. As was stated earlier, if leaves are immersed in water for an hour or two, a point is reached when no more moisture will be immediately taken up upon further wetting, or it will be taken up so slowly as not to be detected with the method used. If, however, these leaves are exhausted of their air

under reduced pressure, more moisture will be absorbed, or if the leaves are left in water for a day or two the same thing will happen. While the experiments are not conclusive as to whether development of ascospores will go on in leaves which are entirely saturated, they indicate that a comparatively high moisture content is necessary for the most rapid maturation.

TABLE 8.—*Effect of different moisture contents of leaves on the maturation of ascospores of Venturia inaequalis, Madison, Wis., 1926*

Treatment of leaves	Stages of perithecial development after 12 days		
	Asci with delimited ascospores ^a	Asci with mature ascospores ^a	Discharge upon wetting the leaves
	Per cent	Per cent	
Wet constantly			
1	16	3	None
2	0	0	None
Moist constantly			
1	71	63	Fair
2	86	62	None
Alternately wet-dry			
1	90	63	Fair
2	80	30	Fair

^aPercentage of total number of asci observed which contained delimited or mature spores. The more advanced perithecia were picked from the leaves for these examinations.

RELATION OF THE AGE OF THE LEAF TO THE PRODUCTION OF THE ASCIGEROUS STAGE

For two years the youngest terminal leaves have produced perithecia as early and in essentially the same abundance as the oldest terminal or spur leaves. The writer has found no evidence that the age of leaves affects their relation to the production of the perfect stage.

SUMMARY

1. The present work has been concerned primarily with the ascigerous stage of *Venturia inaequalis* (Cke.) Wint. in relation to some of the more important factors which influence its development. Special consideration has been given to the effect of these factors on the production of a timely and abundant ascosporic inoculum for the initiation of apple scab epidemics.

2. Penetration of the interior of the leaf by hyphae from the subcuticular stromatoid layer of the fungus and the production of perithecia take

place at any point beneath the indefinitely margined lesions, while these developments take place, in the main, only at the periphery of the more definitely margined lesions. The failure of the fungus to penetrate from the cells of the stromatoid layer near the center of the latter type of lesion seems to be correlated with the lack of protoplasmic contents in these cells. There seems to be an inverse relationship between the number of conidio-phores produced from a given area of the stromatoid layer and the presence of protoplasm in the cells of this area.

3. Evidence was not obtained that hyphae of the fungus ramify the tissues of infected leaves extensively after leaf-fall and produce perithecia at points remote from lesions. Perithecia were rarely found more than one centimeter from a scab lesion.

4. Lesions which had been macroscopically invisible while the foliage was green were often noted after the leaves had become yellow. This may account, in part, for the conception that the hyphae of the scab fungus ramify the interior of the leaves extensively.

5. No indication has been found that the fungus penetrates the tissues of uninfected leaves on the ground and there produces perithecia.

6. The results of the studies reported above indicate that the quantity of perithecia produced in overwintered leaves bears a direct relationship to the type and abundance of lesions on the leaves.

7. Studies of the development of perithecia and descriptions of the grosser stages by which this development may be traced are reported.

8. Delay in the time of leaf-fall is accompanied by delay in the time the ascospores reach maturity the following spring. This delay of maturation of ascospores is not, however, commensurate with the delay in leaf-fall.

9. Meteorological factors, cultural practices and soil, the scab disease, and varietal differences of the apple appear to be important in inducing variability in the time of leaf-fall.

10. While perithecia developed in the leaves of all the apple varieties studied, they appeared to mature earlier in the leaves of some varieties than others.

11. Hyphae penetrated from the subcuticular stromatoid layer into the interior of the leaves at temperatures ranging from 4° to 28° C.

12. The optimum temperature for initiation and early growth of perithecia in oatmeal agar, agar made from dead apple leaves, and naturally infected dead leaves was near 13° C. Certain differences which were found when the fungus was grown on malt extract agar are discussed. On apple-peel and oatmeal agars short exposures to temperatures near 0° C. appeared to stimulate perithecial initiation, while this was not noted when the fungus was grown on apple-leaf agars. Limited data strongly suggest that perithecia may be initiated at temperatures below 0° C. These data are, however, not conclusive.

13. The optimum temperature for maturation of ascospores appears to be near 20° C. Temperatures of 24° C. and above are detrimental to normal ascospore development if the leaves are moist. No deleterious effect was observed, however, when air-dry leaves were kept at temperatures above 24° C. for eight days.

14. There appears to be a transition from a lower to a higher range of thermal requirement as the perithecia develop. This phenomenon is discussed in relation to the shortening of the period from leaf-fall to maturation of ascospores when leaf-fall is delayed.

15. Minor consideration was given to the absorption and retention of water by dead leaves of different varieties of apples and the possible relation of these two factors to the production of perithecia in the leaves.

16. Hyphae were not found to grow from the subcuticular stromatoid layer into the interior of air-dry dead leaves, but such growth took place readily when the leaves were moist.

17. Perithecia were initiated as readily in leaves which were moist enough to be fully pliable as in leaves which were wet.

18. Perithecia matured more rapidly in leaves which were alternately wet and dry than in leaves which were continuously wet. On the other hand, when the leaves were moist enough to be fully pliable, the perithecia matured as rapidly as those in leaves which were alternately wet and dry. However, continuous moisture led to certain abnormalities in perithecial development.

19. Young terminal leaves produced perithecia as readily and in apparently the same abundance as the older spur or terminal leaves.

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EXPLANATION OF PLATES

PLATE V

Sections of perithecia of *Venturia inaequalis* in various stages of development in apple leaves.

A. Perithecial initial, showing origin from coiling of hypha ($\times 382$).

B. Formation of ascogonium from ascogonial initial which is at first straight but later becomes curved ($\times 535$).

C, D. Perithecia before trichogyne has been formed. Ascogonia one to several separate ($\times 535$).

E. Formation of trichogyne by elongation of one end of ascogonium which pushes through wall of perithecium ($\times 535$).

F. Perithecium with antheridium near it ($\times 125$).

G. Perithecium with long trichogyne ($\times 535$).

H. Ascogonium beginning to disappear. At this stage hyphae begin to appear in the lumen of the perithecium ($\times 522$).

I. Perithecium just before the appearance of the ascogenous hyphae. The remains of the ascogonium appear at the base of the perithecium and the remainder of the lumen is filled with hyphae ($\times 352$).

J. Somewhat later than I. A few ascogenous hyphae have appeared at the base of the perithecium ($\times 618$).

K. Appearance of asci. The ascogonium has entirely disappeared ($\times 518$).

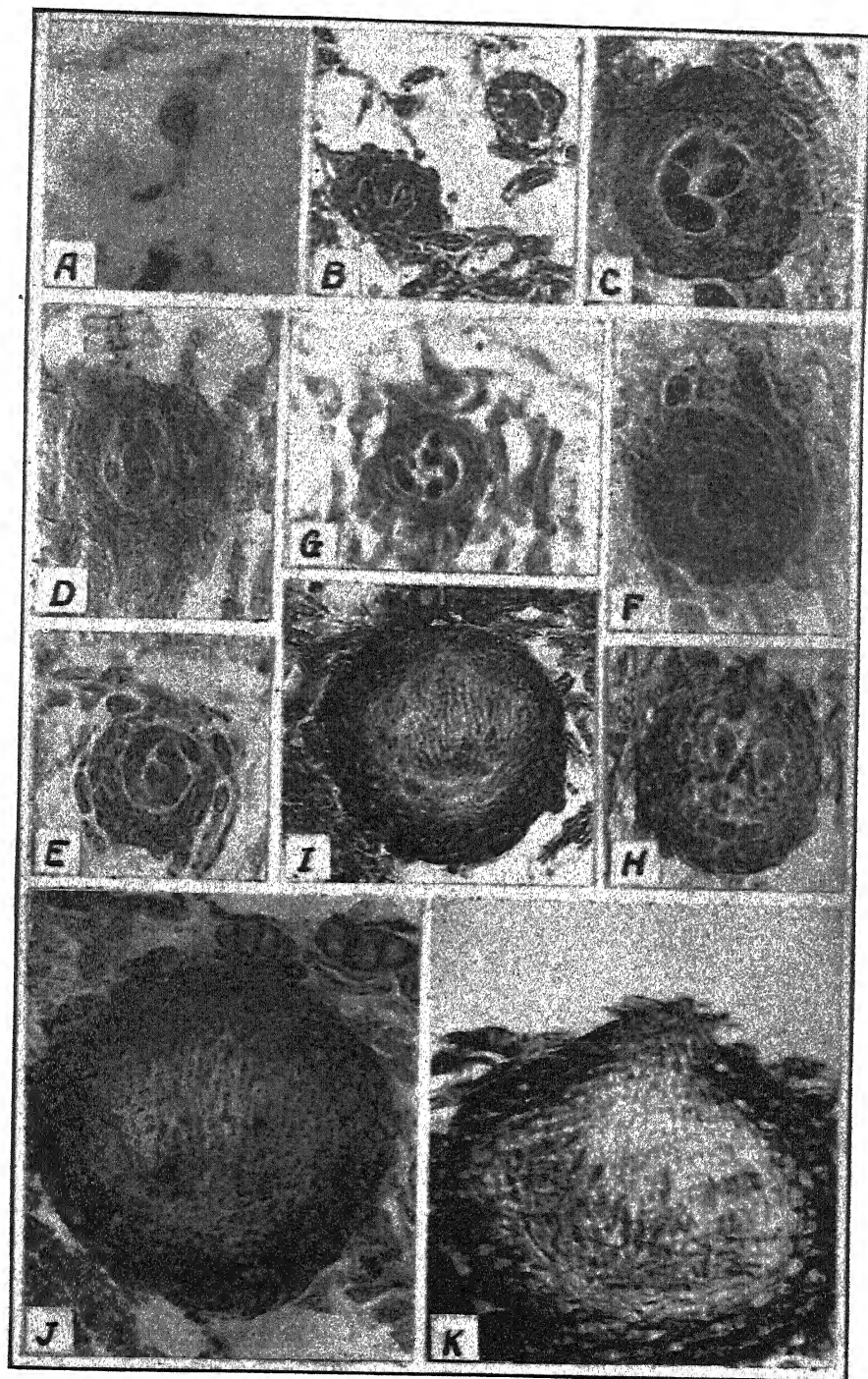
PLATE VI

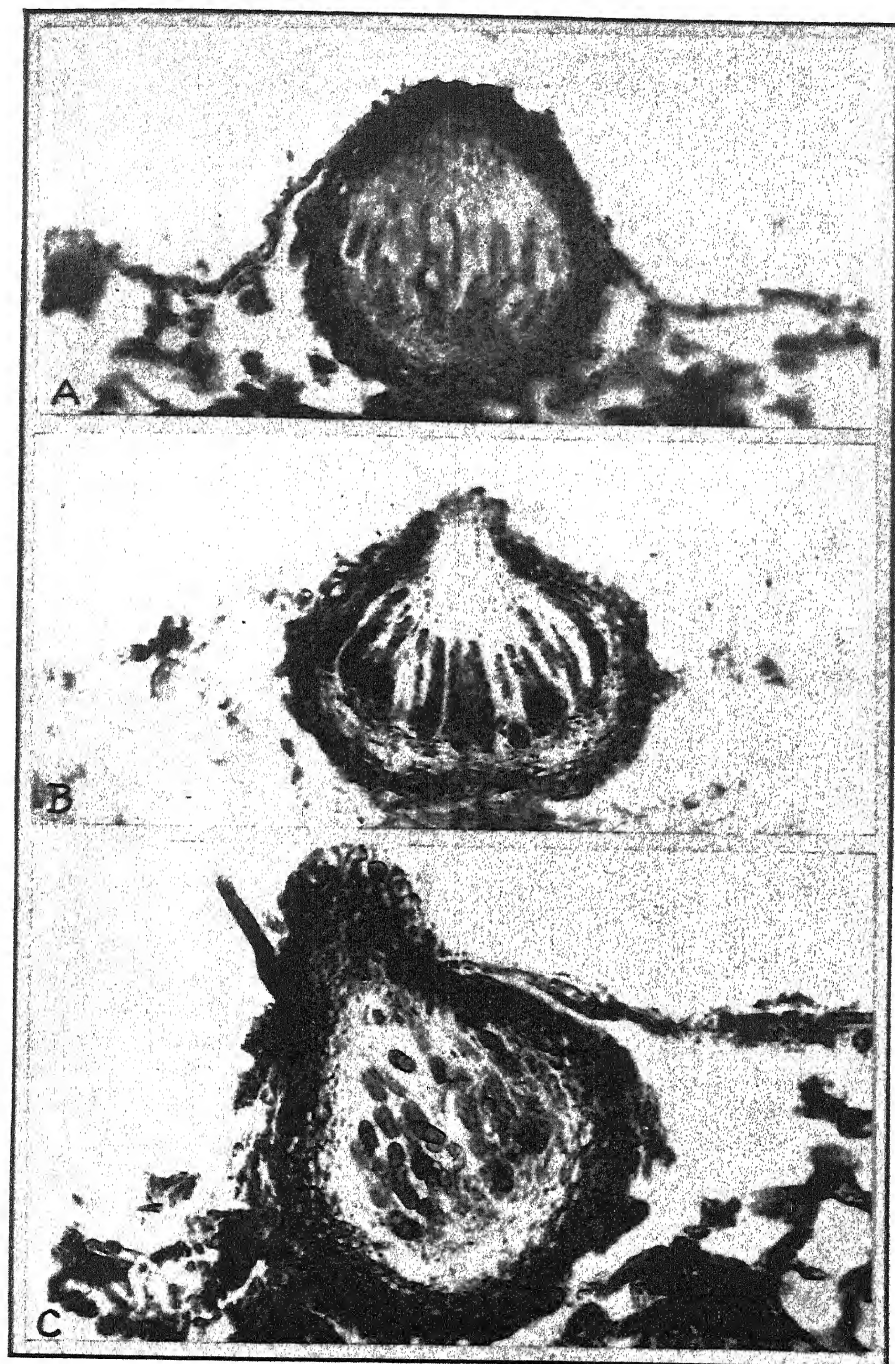
Sections of perithecia of *V. inaequalis* showing later stages of development in apple leaves.

A. Asei about one-half mature size. Hyphae are still present in the lumen of the perithecium ($\times 368$).

B. Delimitation of ascospores ($\times 368$).

C. Mature ascospores. The hyphae have entirely disappeared from the lumen of the perithecium ($\times 560$).





A CONSIDERATION OF THE PATHOGENICITY OF THE COTTON-WILT FUNGUS, *FUSARIUM VASINFECTUM*¹

H. R. ROSEN

Attention was recently (12, 13) called to the fact that the growth of the cotton-wilt fungus, *Fusarium vasinfectum*, on a medium containing nitrate led to a reduction of this substance into nitrite. It has also been shown that nitrites in quantities comparable to the amount found in the nutrient media are extremely toxic to cotton.

Assuming these statements to be correct (the writer realizes that a great deal more quantitative work is necessary and that other factors may be involved in the use of nitrate), what would be the effect of adding nitrate to a soil which is poor in nutrients and in which the cotton-wilt fungus predominates? To answer this question, a series of soil experiments was undertaken involving the use of Ottawa silica sand and of a light sandy loam soil. The first, a whitish quartz material, was utilized because of its relative freedom from any active chemical ingredients, and the second to enable a direct comparison with soils which are frequently infested with the wilt-producing fungus.

Altogether the series consisted of 24 jars, mostly of a one-gallon capacity. With some variations, which do not concern us for the present, each treatment was triplicated and the results here given represent the averages of the various trials. The variety of cotton seed utilized was a strain of Trice that is very susceptible to wilt. A rather heavy inoculum was mixed thoroughly with the sand and soil, and a somewhat large amount of sodium nitrate was used, though not in sufficient quantities to produce injury. The amount added to each jar approximated about 1,000 pounds to the acre. Table 1 gives the percentages of germination obtained.

The outstanding result of these tests is the complete suppression of germination in all pots in which NaNO_3 existed in the presence of the fungus. (See Figs. 1 and 2.) This is true not only of the pure quartz tests, but also of the sandy loam ones, and it appeared in sterilized as well as in unsterilized soil.

The seeds, the same number in each jar, were planted at the same time, on July 1, immediately following the application of the inoculum. The latter, as previously noted, was used in relatively large quantities, the con-

¹ Research paper No. 82, Journal Series, University of Arkansas.

TABLE 1.—The percentages of germination obtained with cotton seed grown in soils infested with *Fusarium vasinfectum* and fertilized with NaNO_3

Type of substratum	Percentage of germination
White sand plus inoculum	80
White sand plus inoculum plus NaNO_3	0
White sand, no inoculum	73
White sand, no inoculum, plus NaNO_3	73
Sandy loam plus inoculum	57
Sandy loam plus inoculum plus NaNO_3	0
Sandy loam, no inoculum	64
Sandy loam, no inoculum, plus NaNO_3	60

tents of a 500 cc. Erlenmeyer flask for each jar. Within three days, germination had started in some of the jars, and within a week practically all germination had ended. Notes, however, were taken up to 21 days following planting. The work was conducted in a greenhouse where the temperature fluctuated, but the fluctuations in temperature were not so great perhaps as would be experienced in the same period out of doors. Roughly speaking, the temperature averaged about 28°C ., varying from about 25°



FIG. 1. Ottawa silica sand experiments. The two lower jars were heavily infested with *Fusarium vasinfectum*, the two upper were free from the fungus. The two jars at the right received nitrate of soda, while those at the left received none. Note the complete failure of germination in the infested pot which contained nitrate. Photographed two weeks after seeding.

to 30° C. The early afternoon temperature, depending on the brilliancy of the sunlight, was apt to be higher than that out of doors, while the night and morning temperatures were also somewhat higher. Owing to the fact that the period in which this experiment was conducted had a high proportion of cloudy days, extremes of heat were not experienced. As the local water supply was heavily chlorinated, distilled water had to be used for watering. No effort was made to run the tests more than three weeks, as the primary object was to obtain the data on germination, which were, by the way, not entirely unexpected.

The experiment clearly brings out the relationship of the cotton-wilt fungus to the germination of the seed, and the marked difference in percentage of germination due to the presence or absence of suitable quantities of nitrates in the soil. In other words, given the same kind and relatively the same quantity of fungus in the soil, cotton seed may or may not germinate, depending upon the chemicals present in the soil. These results may in part, at least, explain the frequent unevenness and lack of stand of cot-

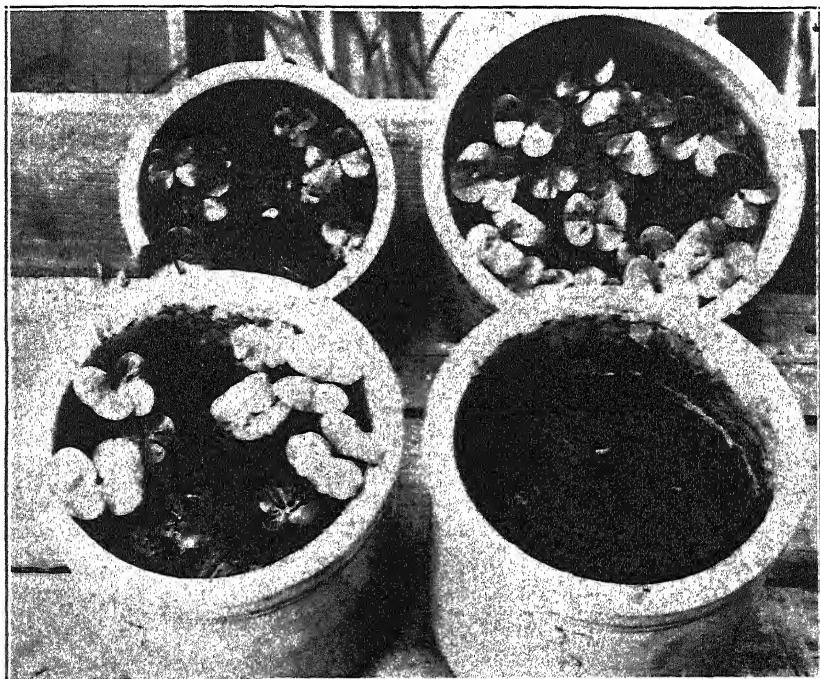


FIG. 2. Sandy loam soil experiments. The jars are directly comparable to those shown in Fig. 1. Here also germination was completely inhibited in the infested jar containing nitrate of soda, the lower right one. Photographed the same time as Fig. 1.

ton, though it does not answer the question completely. It should be noted that the replanting of cotton two or three times in one season is not confined to seasons of floods; it is all too common in ordinary seasons. Also, the huge quantities of cotton seed planted, necessitating a large amount of labor in "chopping" or thinning, are required partly on account of the relatively high mortality of cotton seed under varying field conditions.

This is not the first time that attention has been called to the possible action of the cotton-wilt fungus in preventing germination. In a description of some field experiments (11) undertaken about four years ago, it was noted that certain fertilizer plots showed stands far superior to adjoining unfertilized plots, but that such superiority existed only where potash alone was used and was unnoticed where a complete fertilizer was present. The whole field, about one acre in size, had been heavily infested with pure cultures of the cotton-wilt fungus, the infestation being so great that "within a few days after planting and before the plants had appeared, a heavy fungous growth could be clearly seen across the field, marking the paths where the cultures had been applied." The explanations then offered for the better stands on the potash plots and for the poorer stands on plots with complete fertilizer were purely conjectural. The work here recorded offers a better basis for an explanation, and it is as follows.

In the presence of the fungus, those plots which had received a complete fertilizer had a lower percentage of germination because of the presence of the nitrate of soda (at the rate of 120 pounds to the acre); and it is quite conceivable that wherever the concentration of nitrate and its reduction product, nitrite, was great enough, lack of germination was encompassed. Of course, in addition to the toxic action of nitrite, there is still the possibility that under certain conditions potassium salts, by influencing the metabolism of the host plant, may act as preventives of reduced stands or of wilt development. The belief that wilt can be controlled by the use of potash is tenaciously held by some planters, although Orton's work (10) and that of Smith and Lewis (17) seem to disprove it. A great deal more work is required under various soil conditions before it can be entirely rejected.

The early work of Appel (1) and Schikorra (14) on *Fusarium* diseases of certain legumes is worthy of attention in connection with the studies here recorded. Appel found that if healthy seed of field peas are inoculated with a spore suspension of *Fusarium vasinfectum* var. *pisi* van Hall, or if such seed are planted in infested fields, germination is inhibited. If seedlings develop, they become either immediate victims of the fungus or wilt later on. He finds the same thing true for the *Fusarium* disease of Lupines; but in *Vicia faba*, on the other hand, attacks by *Fusarium* are

limited in the seedling stage to parts of the cotyledons, and it is only in later stages of the development of the plant that the disease takes hold by means of the vascular bundles. Again, Appel notes that weak plants of *Vicia faba* and of *Lupinus angustifolius* are subject to a regular foot-rot, "Fusskrankheit," which kills the plant by means of the death of the lower part of the stem. The writer finds the same thing true for *Fusarium vasinfectum* acting on cotton seedlings when a heavy inoculum is used in the field or in pots kept in the greenhouse. Other factors such as temperature, moisture, and type of substratum also play an important rôle in the development of damping-off or foot-rot, caused by *Fusarium vasinfectum*. Elliott (5) has called attention to the marked influence exerted by the medium used for growing the cotton-wilt fungus in influencing the development of other organisms which may attack the cotton seedlings and produce damping-off. Cornmeal or cottonseed meal, when incorporated into the soil even in small quantities, "made so favorable a medium for fungous and bacterial growth that plants in both the control and inoculated pots were killed by almost any organism which chanced to infect the meal." Now *Rhizoctonia* in particular is often to be found causing damping-off even in sterilized soil, for, unless extreme precautions are taken, it takes but a relatively short exposure to ordinary greenhouse conditions for it and other soil inhabitants to invade the sterilized soil. Parenthetically it may be noted that sterilizing the soil by heat or by chemicals is apt to change the biological, chemical, and physical qualities of the soil to such an extent that comparisons with field behaviors of the same soil, and of any parasite associated with it, must be made with considerable caution. The point is, that while Elliott is doubtless correct in regard to the possibilities of various fungi and bacteria, other than the cotton-wilt pathogen, causing damping-off, nevertheless, *Fusarium vasinfectum* can also produce a cortical root-rot or a rot of the subterranean portions of stems, and this may manifest itself as a typical damping-off. Indeed, in the same publication he notes that, in some cases, plants growing in soil artificially infested with *Fusarium vasinfectum* died without showing positive indications of wilt but merely rotted off at the soil line, and very recently Fahmy (6) described a girdling of the hypocotyl of cotton seedlings grown in soil heavily infested with the wilt-producing organism. Further than this, it may be stated that the type and quantity of inoculum as well as the kind of soil has a very marked influence on the severity of infection and on the type of infection. More will be said of this in a later publication.

THE RELATIONSHIP OF FUSARIUM VASINFECTUM TO CORTICAL ROOT-ROT

It has been a common observation of various investigators that the cotton-wilt fungus in artificial infection experiments often fails to produce a

true wilting. Erwin F. Smith (16) noted it, and in his usual painstaking way undertook large numbers of artificial infections, but while he was successful in producing the *Fusarium* wilt of watermelons, he met reverses with the cotton pathogen. He says, "All of the cotton plant inoculations have failed. These also were soil inoculations, and were performed on many small plants, using the cotton fungus, the cowpea fungus . . . , and the melon fungus. The experiments were repeated in different years and were continued in some cases for a long time with both sea island and upland cotton." Even Atkinson (2), who was the first to discover and name the causal agent, had difficulty in inducing healthy plants to wilt. He tells of having isolated the fungus in pure culture and attempting artificial infections and says, "The *Fusarium* was considered not to be a sufficiently aggressive parasite to be able to make its way into the ducts of the circulatory system unaided. Having found that the damping-off fungus could disease the stems of the young cotton, and that many plants even when the ulcer reached the circulatory system recovered from the effects of this external injury, it was suggested that possibly this fungus could open the way for the entrance of the *Fusarium*." He then inoculated some young plants with the damping-off fungus (probably *Rhizoctonia*, judging from his description of the mycelium), and when some of these had contracted the disease so that "an opening was made in as far as the vascular tissue, a portion of the earth was removed and pure cultures of the *Fusarium* were placed directly against the diseased portions of the plants. . . . One plant about 12 inches high died exhibiting signs of the disease in one leaf," and he found by microscopic examination that the *Fusarium* occupied the ducts near the ground. In another single case he succeeded in producing wilt by placing stems of diseased plants near plants suffering from attacks of damping-off, and he remarks, "The diseased condition of the ducts (in plants that show damping-off) affords an opportunity for the fungus to gain a foothold." Orton (9), in 1900, was the first to produce artificial infections in a fairly adequate number of plants, and his views concerning the aggressiveness of the parasite, as then expressed, are diametrically opposite to those held by Atkinson. He says, "It is not believed by the writer . . . that the assistance of the root nematodes or any fungus is necessary to allow the wilt fungus to gain entrance to the roots of cotton. . . . The indications are that the fungus is a sufficiently aggressive parasite to make its way unaided into the vascular system of the plant whenever the plant is liable to infection." He obtained 14 successful infections out of 24 plants inoculated with pure cultures. Of these 14, 7 showed the typical wilt form of the disease, while all the check plants remained healthy. "That a larger proportion of the inoculations did not succeed is believed to be due to the

small amount of fungus used and to the natural resistance of the plants." Smith's failure to obtain infections he believes was due either to the slow growth of the plants or to their natural resistance.

It is a very difficult thing to believe that all of Smith's failures and those of almost every investigator who has dealt with this disease are due to the causes assigned by Orton, although, as will be shown later, natural resistance is possessed by most cotton varieties. Smith succeeded in producing infections with the watermelon *Fusarium*, and it is quite probable that the amounts of inoculum he used with this were comparable to the amounts he applied to cotton inasmuch as the experiments on these diseases were conducted at the same time. Again, Smith used a number of varieties of cotton and failed with all. The present writer has conducted relatively large numbers of experiments over a period of about seven years, involving field and greenhouse tests, using at times over 50 different varieties and strains, many of them extremely susceptible under certain field conditions; and with very few exceptions the typical field forms of wilt were not obtained. Some of these experiments will be detailed below. Elliott (5) likewise failed at times to obtain typical infections and attributed his failure to a variation in the pathogenicity of various strains of the cotton-wilt fungus. Very recently Singh (15), in India, made a careful study of a number of strains of *Fusarium* isolated from cotton plants naturally infected in the field and from various types of wilt-infested soils, and he was unable to obtain typical wilt by means of artificial infections. His conclusion with reference to the few cases where he obtained wilt is that the fungus "is not a virulent parasite on cotton plants, since it was found in plants, both in the inoculated and in the uninoculated series, only when the plants were attacked by *Rhizoctonia*." Of course the meaning of the term "virulent parasite" is not fixed and may be used variously. If Singh means that this organism cannot attack and produce disturbances of varying degrees of severity, then his statement is of doubtful significance. But if he means that *Fusarium vasinfectum* can only produce wilt under restricted conditions and that it is a wound parasite, requiring a nidus of dying or dead host cells before it can injure the living tissues, then the writer is in full agreement with him, having presented this view in a previous publication (13). Butler's (3) recent publication again calls attention to the fact that artificial inoculations are not readily obtainable with the cotton-wilt parasite; he believes that the organism is capable of definite pathogenic action only under certain conditions. These, he further believes, have not yet been elucidated and are probably not connected with the composition of the soil, as Dastur (4) has concluded. The latter has proposed the idea that wilting is related to the presence of iron and aluminum compounds in the soil, inasmuch as he was unable to produce wilt by artificial infections with the fungus which

had been associated with wilted plants. On the other hand, Fahmy (6), perhaps unintentionally, gives the impression that wilt may be produced at will by artificial inoculations; at least nothing is said about any failures to produce wilt artificially in susceptible varieties.

By what means does the organism penetrate and what are the first signs of the disease it produces? In the pot experiments recorded in the first part of this paper an excellent opportunity was afforded to study the invasions of the parasite, particularly in those jars where pure, quartz sand was used. This material does not stain the roots as ordinary soil does, and any lesion, even though it be small, is apt to appear conspicuous against the whitish background of the uninfected parts. In the jars which had not received nitrate and in which the fungus was incorporated, every plant, when carefully removed, showed a very large number of brownish lesions of varying sizes on the roots, while control plants in uninfested jars showed very few or none at all. A relatively large number of lesions, when examined under the microscope, showed the presence of the fungus, in some instances with the typical microconidia of *Fusarium vasinfectum*. In other cases the fungus was not observed, and the chances are that it had either perished or was obscured by the entrance of secondary invaders. Occasionally bacteria and other fungi were seen in such lesions. When surface sterilized and plated out on potato dextrose agar, the fungus was isolated in about 25 per cent of the cases, while the remainder were sterile or developed various types of microorganisms, often producing both bacterial and fungous growth. This, of course, is to be expected under the conditions prevailing about a root system. A careful histological study has not been made, but there was fair indication in about 50 per cent of the cases observed of a suberization process going on; and the probabilities are that, if the material had been embedded in paraffin and carefully sectioned and stained, the production of corky layers would have been detected in a greater percentage of the lesions.

One of the most significant findings with reference to this discussion was the frequency of lesions at the collar of roots, that is, in those natural wounds made by the passage of extruded, secondary roots. In very few instances were discolorations observed which did not center around wounded areas, and even in these instances it was difficult to tell with certainty if wounding of some sort had not occurred prior to the advent of the fungus. The general view presented by an infected root system under the conditions of these experiments was that of the action of a wound parasite, which in a large number of instances was barely able to gain a foothold on the host and produce more or less localized, superficial, cortical discolorations.

In addition to the large numbers of small cortical lesions, another effect noted was the lack of elongated feeding rootlets, particularly in the lower

parts of the system. The result was that the root complex usually consisted of one elongated tap root with a mat of short, feeding roots, bunched more or less together at the upper part of the root. It was quite obvious that the plant had lost a large number of the finer roots and that the bunching represented successive efforts of the plant to replace the roots that were being destroyed. As the control plants were under the same environmental conditions, other than not being in the presence of the fungus, there is very good reason to suppose that the symptoms here described are directly or indirectly due to the action of the introduced parasite. In addition to this evidence, the fungus has been located in a number of such decayed roots by microscopic observations and by pure culture isolations. This effect on the roots has, with very few exceptions, been entirely overlooked by students of this disease and by textbook writers, although Orton (9) called attention to it in 1900. He noted that tufts of roots grew from points of infections and that "several short roots would start from a place which would normally have produced one longer branch." Many of these smaller roots, he found, were killed by the fungus, and he considers the stunted top growth of such plants as due to the action on the roots. He found such root tufts both in artificial infections in the laboratory and on plants growing under natural field conditions. With reference to the latter, it is of considerable interest to note that these were observed in fields where Orton apparently did not find any typical wilted plants. For, he says, the presence of such malformations "on the roots demonstrates the presence of the wilt fungus in the soil, even when the amount is so small that no harm is visible aside from the reduced growth of the plants."

The writer's studies up to the present are not sufficient to indicate where and how infections started on these rootlets. Very frequently the discoloration and rotting is to be observed at or near the tip, and there is some reason to believe that the infections started when the roots were in the incipient stage, shortly after, or at the time when they broke through the overlying cortical tissue of the mother root. Inasmuch as the region around the opening made on the mother root by the passage of the rootlet is often infected, as previously described, it is quite possible that the fungus may have passed along from the wounded tissue of the mother root into the developing rootlet. There is also the possibility that the region of the root tip with its dead and dying cap cells offers abundant opportunity for the fungus to gain a foothold there, whence it spreads to the adjoining healthy tissue. It is important to note that, in spite of such numerous injuries produced by the fungus, it usually does not penetrate sufficiently into the vascular system to produce wilt.

THE ARTIFICIAL PRODUCTION OF WILT BY *FUSARIUM VASINFECTUM*

It has already been fully recorded that the production of a true wilt, comparable to that observed under natural conditions, is frequently unobtainable in artificial infections. By natural wilting in this disease is meant the loss of turgescence of foliar tissues and of succulent portions of stems, resulting in a drooping, withering, discoloration, and premature death of the plant. It should be distinguished from those symptoms induced by the same parasite resulting in various forms of root injury, described previously, which are more or less localized and which may result in a stunting of the whole plant or some of its parts. Likewise, it is to be distinguished from those forms of the disease in which no true wilting is involved, and in which the plant continues to live for relatively long periods, often up to the first killing frost, and also in which the disease may manifest itself as a more or less limited, often narrow, thread-like discoloration of parts of the vascular system. The latter is frequently obtainable in artificial infections when very little or no true wilting is to be had, and it not infrequently happens that the plantings of portions bearing the discolored tissues remain sterile. Most of these internal discolorations do not appear until the plants are quite advanced in age, after a period of 60 to 90 days, and at the end of this time plants grown under ordinary greenhouse conditions are apt to be so weak and debilitated that one hesitates to ascribe any marked degree of virulence on the part of the fungus or on the degree of tolerance possessed by the host plants.

It may be worth while to describe some of the attempts at artificial infections in field and greenhouse experiments, undertaken primarily to test the degree of resistance to wilt by different varieties of cotton. A field approximately one acre in size, consisting of a rather heavy clay or silt loam soil, near Fayetteville, Arkansas, was heavily infested with pure cultures of a *Fusarium* which had been isolated from a badly wilted cotton plant. It is the field which has previously been cited (11) as showing a large amount of seedling damping-off and lack of germination, both of which have been ascribed to the fungous inoculum. The varieties used were Webber Deltatype, Express, Pedigree Register, commercial Acala Number 5, and Dix-Affi. Of these, Dix-Affi, and Express to some extent, are known to be resistant to wilt. The others, on the other hand, have been found to be susceptible to wilt under various conditions in Arkansas. Of the hundreds of plants used, only 18 developed a true wilt: 9 of the Webber Deltatype, 8 of the Express, and 1 of the Dix-Affi. The wilting was noted in the period between June 12 and June 26, when the plants were about six to eight inches high, and the appearance of the vascular systems, as well as the isolations from them, left no doubt as to the type of disease. On the

other hand, all of the remaining plants showed no recognizable symptoms until late in the growing season, in September. At about picking time, when the plants were pulled up and their interiors examined, approximately 25 per cent showed linear discolorations of the bundles, involving all of the varieties. But, so far as yield is concerned, no appreciable difference could be detected between plants without interior discolorations and those with discolorations. The appearance of the plants was such that it was considered worthless to obtain data concerning yields of infected and uninfected plants. As far as gathering data concerning varietal resistance and susceptibility went, the experiment was at that time considered quite a failure. It is quite interesting to note in this connection that, while the cotton failed to wilt in this artificial soil infestation, there was almost 100 per cent wilt in susceptible varieties of tomato in a field not more than 150 yards away from the cotton field, also artificially infested with the wilt-producing organism, *F. lycopersici*.

Since the experiment detailed above was conducted outside of the main cotton-growing section of the state, it was thought advisable to utilize another field, within the cotton area. In 1923, an acre field near Van Buren was obtained which was so badly infested with wilt that about three-fourths of the plants were counted as wilted in 1922. The soil varied from almost a pure river sand to a sandy loam, and in years of high water the Arkansas River, which flows close by, covers a considerable part of the field. In the fall of 1922 it had been planted to winter oats, and when it was obtained in May of the following year the oats were turned under without being cut or harvested. In order to insure thorough soil infestation, the cotton seeds were dipped in a very heavy spore suspension, and in addition were given another spore dose, by means of a sprinkling can, as they lay in the furrow. The soil was then immediately raked over them. About 30 different varieties and seeds from 150 selections, representing disease-resistant plants, were grown. It would be burdening the record to list all of the varieties, but there were included commercial types of Acala, Rowden, Trice, Webber, and other varieties that are usually considered susceptible. Throughout the growing season only six wilted plants were observed, and this in spite of the fact that cotton wilt was quite common in the vicinity of this field. The experiment was repeated on the same field for three successive years, with approximately the same result. Each year a heavy inoculum was applied; in one case it consisted of a mixture of various strains, the fungus being applied as a spore suspension or as spores and mycelium growing on cotton stems. The amount of inoculum used in any one year was several times as great as that necessary to insure thorough soil infestation with the tomato wilt *Fusarium*. In 1924, nine wilted plants appeared, and in 1925 only six

plants wilted. Obviously such numbers are of little or no value in indicating resistant and susceptible varieties.

In 1925, in spite of three years of artificial infestations, the yield of cotton was more than a bale to the acre. It has been reported by a number of investigators that the cotton wilt fungus is capable of living in the soil for a number of years in the absence of a suitable host. If we assume that the cultures used for inoculum were non-pathogenic, it would still be necessary to explain the absence of wilt in a field which had been so thoroughly infested less than one year previously. Likewise, it does not seem proper to attribute this failure to lack of proper temperature, either soil or air, for, as previously indicated, adjoining fields in the section of the state around Van Buren showed various amounts of wilt in all three of the years, and large numbers of successful artificial infections have been obtained with *F. lycopersici* on tomato in the field and greenhouse, with the production of typical wilt, when very little or no wilt was obtained with the cotton pathogen, tried at the same time. Furthermore, Jones, Johnson, and Dickson (7) have concluded that all agricultural soils "tend to approximate the same temperature under the same meteorological conditions." It therefore seems reasonable to assume from these tests that cotton wilt may be more conditioned by the chemical, physical, and biological soil factors than are certain other *Fusarium* diseases, such as cabbage yellows, flax wilt, and tomato wilt. It will be recalled that tomato plants are like cotton plants in that they require rather high temperature for good growth. Is it possible that the growth of the oat crop and its use as a manure, in the interval between the successive crops of cotton, may have so changed the soil conditions as to render the plants more or less immune to wilt? More will be said of this in a future publication.

The artificial production of wilt in the greenhouse has sometimes been accomplished, but mostly the tests have been failures. There is evidence that part of these failures was due to the lack of virulence of some of the strains utilized for inoculum (See Figs. 3 and 4); and there is always the possibility of attenuating the parasitic habit of any microorganism which is grown on artificial culture media. On the other hand, the history of the parasite, and the length of time it may exist in the soil under field conditions, do not warrant the assumption that it loses its parasitic habits readily. Again, it seems too much to assume that such able investigators as Erwin F. Smith, G. F. Atkinson, and others did not have the true parasite or that their work was not otherwise carefully done. As a further check on the possibility that the true parasite was not at hand or that it became non-infectious after its isolation, soil from various parts of the State was obtained in which cotton had wilted badly, about eight weeks previously,

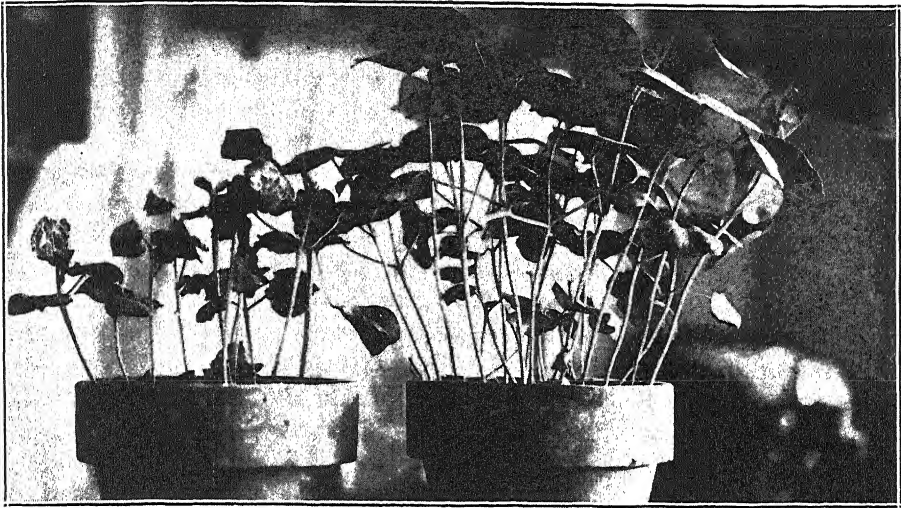


FIG. 3. A comparison between the pathogenicity of a strain of *Fusarium vasinfectum* isolated from a wilted cotton plant obtained in Arkansas (at the left) and one obtained from a wilted plant grown in Louisiana (at the right). The same number of seeds were sown in each pot, and the pots were otherwise under comparable conditions. The Arkansas strain had wilted a large proportion of the plants when the Louisiana strain had produced very little wilt.

under natural field conditions. Sufficient soil was obtained to enable the growing of several hundred plants. The greenhouse temperature under which they were grown was kept fairly well regulated, at a temperature of around 25° to 28° C., and although considerable seedling damping-off developed, there was very little true wilt. The plants were kept growing for about 40 days and then discarded. Less than 10 per cent had developed wilt, in spite of the fact that the seed represented a strain of Trice which was very susceptible to wilt under some field conditions.

If *Fusarium vasinfectum* were capable of wilting plants as readily as *F. lycopersici*, where would the American cotton industry be to-day? Cotton for a long time has been grown far more extensively and continuously in the South than tomatoes; and yet the cotton growers, taken as a whole, have paid next to no attention to the necessity of using wilt-resistant varieties, in spite of the fact that cotton wilt has been known in Arkansas, and in the South as a whole, for at least 40 or 50 years. Looking at it from another angle, if cotton wilt were always as destructive as the field photographs presented by various investigators show it to be, cotton growing by this time would have become a lost art. These photographs indicate what may happen in some fields, but they certainly do not indicate the average

condition in wilt-infested cotton fields. One is forced to conclude that this fungus is far more limited in its wilt-producing properties by certain factors than are other *Fusaria*. These factors have not as yet been entirely elucidated, but the work thus far seems to indicate the following possibilities.

It should be borne in mind that the cotton plant is a perennial and has a very firm, rather woody root and stem system. It is easily killed by frost, and consequently does not live more than one season under the usual conditions prevailing in the cotton-growing sections of the United States. Only when the plant is young and succulent is it most liable to serious root and stem injury by various microorganisms, especially *F. vasinfectum*. Therefore, conditions which make for succulence or which perpetuate the juvenile stage render the plant more liable to attack by root parasites. That is the reason, the writer believes, for the greater prevalence of wilt in wet seasons. Conversely, given good growing conditions, with a proper amount and balance of nutrients, even though there may be abundant moisture, the plant, by developing firm, woody tissues, is able to ward off the attacks of various root invaders. In the absence of good soil, or when some agent is

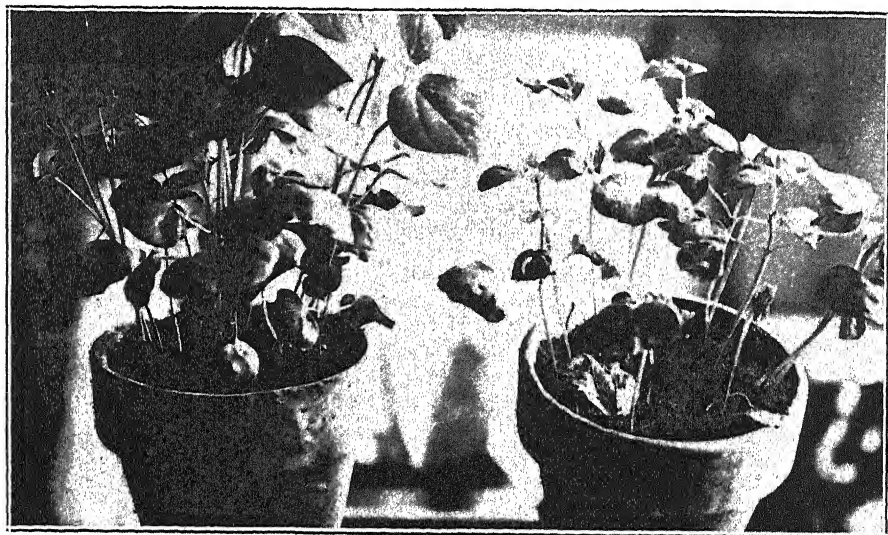


FIG. 4. A comparison between the pathogenicity of a strain of *Fusarium vasinfectum* isolated from a wilted cotton plant obtained in Mississippi (at the right) and one obtained from a wilted plant grown in Texas (at the left). The same number of seeds were sown in each pot and the pots were otherwise under comparable conditions. The Mississippi strain had wilted a large proportion of the plants when the Texas strain had produced very little wilt. The strain from Arkansas was very comparable in this respect to the Mississippi one while the Louisiana and Texas strain were closely related from the pathogenic viewpoint. (See Fig. 3.)

present in good soil which disrupts the tissues or which prevents the formation of wood and cork, the plant is particularly liable to invasion by *Fusarium*.

Thus it is, the writer believes, that nematodes, wire-worms or other insects, Rhizoectonia lesions, water levels which tend to asphyxiate roots, and other factors which destroy or break down root tissues, or which inhibit normal root development, render the plant susceptible to wilt. The action of nematodes on roots is largely that of producing localized, hyperplastic overgrowths, consisting mostly of soft, parenchymatous tissue and a reduced amount of cork and wood. It seems reasonable to assume that this production of soft, succulent tissue offers excellent opportunity for the growth of the cotton-wilt parasite, and this appears to be the explanation for the prevalence of cotton wilt on nematode-infested soil. It is not intended to suggest that factors other than mechanical ones are not involved in the resistance and susceptibility of cotton to wilt. It impinges on the question of parasitism in general, about which very little is known. And, even in the production of cork or wood, which may act as physical barriers, there undoubtedly enters a number of factors, many of them chemical, which must be considered. If we look at the question from the standpoint of ideal host development, we may be justified in assuming that most varieties of cotton are resistant to wilt when conditions are proper for their normal development, and it must be added that this does not necessarily mean the production of very large tops or of many bolls. The standard of normality here involved is the production of a hardy root and stem system, and more specifically perhaps, the inherent property of readily producing suberized layers. It may well be, according to this theory, that in so-called susceptible varieties, suberization of roots under some conditions does not take place as readily as in resistant varieties.

What are the conditions in ordinary pot or jar experiments conducted in the greenhouse? Are the plants more succulent than under ordinary field conditions, or are they more woody? Of course everyone knows that under these conditions there is apt to be an overcrowding of plants and that more plants will be grown than the soil can well support. This becomes more and more evident as the plants grow larger. They appear thin, wiry, more or less spindling, and are anything but succulent. As far as greenhouse cotton is concerned, succulence is relatively rare, except in the early seedling stages; and when it is considered that pains are usually taken to sterilize the soil, thus killing any nematodes, wire-worms, and various micro-organisms which are capable of injuring root tissues and permitting ready access of the cotton *Fusarium*, the frequent failures to obtain wilt artificially are not so surprising. While a great deal remains to be determined about

the conditions which favor succulence in cotton, moisture and nutrients which make for unbalanced metabolism must be considered. The use of a quick acting and readily available source of nitrogen such as nitrate of soda will tend toward succulence unless other soil conditions are proper for the development of firm, woody tissues. Aside from the fact that the fungus is capable of reducing nitrates to nitrites which may kill some of the roots and thus enable the fungus to gain access to the plant, any nitrate which is taken up by the plant and not reduced by the fungus will make it more vulnerable to the action of *Fusarium vasinfectum*. On the other hand, when the soil is in a good physical condition and contains all the elements, properly balanced, for the development of firm, vigorous tissues, then cotton wilt will be a negligible factor. Of course the presence of nematodes or other organisms, or adverse temperature or moisture, which destroy the natural resistance of cotton plants, will tend to vitiate the action of any soil, no matter how good it may otherwise be.

Several months after the completion of this paper and after it had been submitted for publication, Neal (8) presented an extensive article on cotton wilt, including in this a study of various soil nutrients in relation to wilt production. He found, among other things, that when cotton plants were grown in washed sand to which various nutrients had been added a very small percentage of plants was killed by wilt, even after an interval of six months following the application of the inoculum. However, while the number of plants actually wilted was small (only one plant is definitely noted as having died of wilt), yet quite a few showed vascular and lateral root discolorations. From the latter symptoms he concluded that the disease was obtained in all the pots but varied considerably in the different nutrients. The most striking results to the present writer are those pertaining to the pots which received iron and in which the vigor of the plants is noted as being fair or poor. In these the results seem more consistent than in the others, hence more valuable, and clearly show a much greater number of infected plants. In another series of tests he used various amounts and kinds of commercial fertilizers in galvanized zinc buckets, and out of 40 plants subjected to the fungus, 12 became infected, of which 3 had received no potash. From this he draws the conclusion that potash, when used in an 8-4-4 or 12-4-4 formula and at the rate of about 800 pounds per acre, "may have increased the resistance of the plants to infection by the fungus." The results of one year's work on commercial fertilizers at two field stations are also given, and, as Neal notes, are quite inconclusive. Neal's work, taken as a whole, adds further emphasis to the writer's conclusion that the growing condition of the plants, especially soil nutrients, has a great deal to do with the presence or absence of wilt on any par-

ticular field, although it should be noted that it is only one element, potassium, that Neal seems to consider as having an important rôle in this phenomenon.

CONCLUSION

As all the evidence gathered thus far clearly indicates that most varieties of cotton grown in the United States show more or less resistance to wilt when conditions are proper for their normal growth and development, it seems reasonable to conclude that the control of wilt may be obtained by improving the growing conditions. The same result may also be accomplished by the use of certain hardy, resistant varieties which are capable of withstanding unfavorable conditions. There is danger of over-emphasizing the importance of one method of control to the exclusion of the other. Thus, while we have had several outstanding resistant varieties for about 25 years, they have been very little used in Arkansas and in other States, particularly since the boll weevil has come in. Such varieties as Dillon, Dix-Affif, Dixie Triumph, and Cook are relatively late in maturing under the conditions existing in this State. They also possess other undesirable features, such as smallness of boll and a rather low percentage of gin turn-out. There is always the danger, in breeding for a certain character, of letting in other qualities which may not be desirable. Indeed, there is a question concerning the breeding of wilt-resistant cotton which has not yet been answered, namely, is wilt resistance correlated with lateness of maturity? Certainly most, if not all, of the resistant varieties developed up to the present are more or less late. It is of course possible that breeders may be able to overcome this difficulty, but in the meantime it seems to the writer that the other method of controlling wilt is worthy of consideration.

Almost all southern investigators of cotton are agreed that one of the greatest problems concerning the profitable production of cotton is the maintenance of soil nutrients. The continuous cropping of cotton, and burning up of soil organic matter, as a direct result of hot, subequatorial latitudes have so depleted the cultivated soil that profitable production is often unobtainable, even when prices are relatively good. Most unfortunately, from one point of view, the southern farmer learned long ago that cotton responds very readily to commercial fertilizers. Consequently, when plant foods were added to the soil they have usually been in the form of quick acting, readily available, and hence easily used up, inorganic salts. Manure or a green manural crop, such as a legume, is rarely used in the South, compared to the North. Rotation is seldom practiced and when a legume is grown it is usually for hay or pasture. Under such conditions it is quite understandable how a crop which is really not a "heavy feeder" becomes subject to such wide-spread maladies as "rust" and wilt. "Rust"

is undoubtedly a malnutritional disturbance, and the writer has here attempted to show that wilt is also intimately connected with faulty nutrition.

From what has been said concerning commercial fertilizers, it must not be concluded that these are not desirable or profitable. The point is that they are often not sufficient to maintain a good soil fertility, although they may give immediate returns. It is of course well known to practical growers that when organic matter is present in the soil the cotton plant is able to make better use of commercial fertilizers. Good farming from this viewpoint will undoubtedly help in controlling wilt in the United States.

As far as foreign countries are concerned, the writer is not in a position to offer suggestions concerning control measures, because in some of them, particularly Egypt and India, the wilt seems to be prevalent on rich soils, which is rarely true in the United States. He would suggest, however, a much more thorough study of edaphic and climatic factors, particularly soil moisture, in relation to wilt development, as offering a possibility for control measures. It is of course conceivable that countries so widely separated and with different climatic and soil conditions may have developed strains of *F. vasinfectum* which differ not only in morphology but also in pathogenicity, as is indicated by Butler's (3) and Fahmy's work (6). While this may be true, private reports from various Indian pathologists seem to indicate that there also the parasitism of *F. vasinfectum* is limited by growing conditions of the host.

SUMMARY

Previous experiments having indicated that the presence of nitrates in nutrient media leads *Fusarium vasinfectum* to produce substances that are toxic to cotton, a series of greenhouse soil experiments were undertaken and here recorded, involving the use of pure quartz sand and of sandy loam soil low in nutrients.

In the presence of heavy infestations of the fungus, those jars which received nitrate of soda showed complete inhibition of germination, while the two types of controls, jars with equivalent amounts of nitrate but without any cotton wilt fungus, and jars with the fungus but without nitrate, showed no hindrance in germination.

A review of the literature indicates that almost every investigator who has worked with this disease has had considerable difficulty in producing artificial infections, with the production of typical wilt. This has led to a study of fungal penetration and of early disease symptoms.

It is shown that the fungus is primarily a cortical rot-producer of roots and of lower stem parts, and that its chief means of entrance is through wounds. Frequently it is found producing a limited, more or less super-

ficial, rotting of tissue about the natural wounds induced by extruded, secondary roots.

The fungus is also found to cause the death of elongated feeding roots without penetrating deeply enough to cause wilt.

Various field and greenhouse experiments are described in which attempts were made to produce wilt in artificial infections, and it is concluded that this fungus is far more limited in its wilt-producing properties by certain soil factors than are other *Fusaria*.

Succulence and the perpetuation of the juvenile stage are in particular pointed out as conducive to wilt. Factors which make for such plant development, as excessive moisture, and improper or unbalanced nutrients, are considered to be necessary for the fungus to produce wilt.

Likewise, any other agents, such as nematodes, wire-worms, and *Rhizoctonia* lesions, which are capable of disrupting root tissues or hindering their normal development, are found to make *Fusarium* invasion possible.

It is concluded that most varieties of cotton, when grown under proper conditions, are resistant to wilt, and that all observations seem to indicate that good farming practice, involving the use of manure or a green manurial crop, materially helps to control wilt in the United States.

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STRAWBERRY DWARF

A. G. PLAKIDAS

INTRODUCTION

The purpose of this brief paper is to give a pathological description of a rather serious disease of the strawberry plant, which is prevalent in the strawberry-growing district of Louisiana, together with some observational and experimental evidence concerning the nature of the disease. It is recognized that the experimental evidence presented is not final or conclusive, but it is believed that a progress report at this stage of the investigation is justified. It is hoped that this apparently undescribed disease will thus be brought to the attention of other workers, and, in this way, more data will be obtained on its nature and geographic distribution. The writer has not been able to identify this disease with any of the strawberry troubles which have been described in detail, or with any of the more or less obscure root-rots or other strawberry troubles of which mention has been made in annual reports of different experiment stations. The term "dwarf" is proposed as a name for the disease.

DESCRIPTION OF THE DISEASE

The most conspicuous symptom of the disease is the severe stunting or dwarfing of the entire plant, a feature which suggested the name "dwarf." The leaves become greatly reduced in size, and are strikingly deformed. The petioles are short, and the leaflets rather elongated in comparison to their width. The leaflets are decidedly unsymmetrical, usually crinkled, and the margins cup upward in the young leaves and usually downward in the older ones. In extreme cases, the leaflets are reduced to mere rudiments. The older diseased leaves are slightly greener in color and more shiny than the healthy leaves of corresponding ages. The petioles, the veins, and the underside of the young leaflets are often reddish purple in color, though this is not a constant characteristic. Often some of the leaves of a plant are purple, while the rest are green. The affected leaves, both young and old, are decidedly brittle, as compared with the normal ones. Affected plants occasionally become "blind"; *i.e.*, the main bud is killed. Blind plants usually die, but occasionally several adventitious buds may develop from the crown. The leaves developed from these secondary buds are small, and their petioles are usually long and spindling. In general, a dwarfed mother plant transmits the disease to all daughter plants arising

from it, though occasionally runner plants may be found which appear perfectly normal. As far as macroscopic symptoms are concerned, dwarf is distinctly a disease of the top part of the plant, the root systems appearing normal in every respect. In figure 1 is shown a very typical specimen of

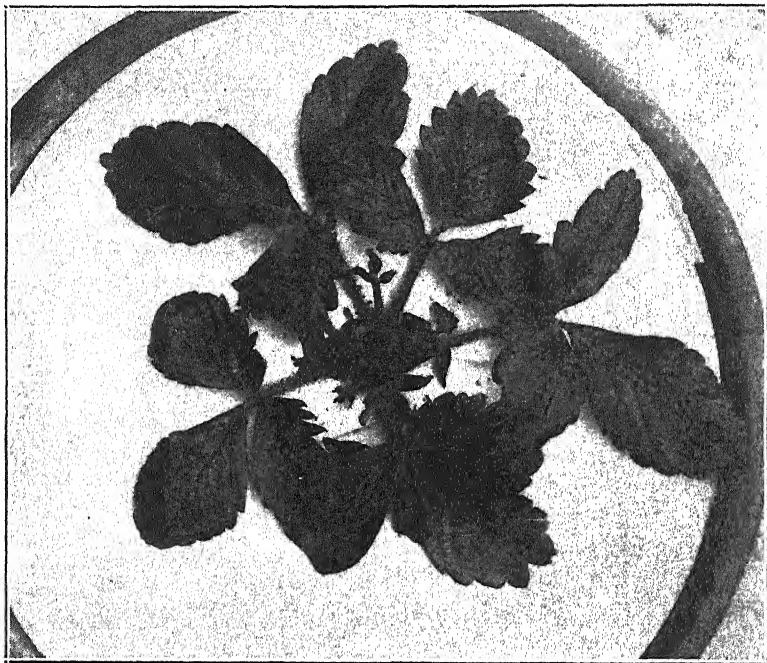


FIG. 1. Young Klondike plant showing typical dwarf symptoms. Note the crinkling of some of the older leaves and the extreme stunting and deforming of the younger ones. 0.7 natural size.

dwarf. The stunted growth of the entire plant, and the crinkled, deformed, and small leaves are clearly shown.

HISTORY AND DISTRIBUTION

It is not known where or when this disease originated. It has been recognized by the strawberry growers in Louisiana for a number of years, and it has been variously designated by such names as "wild plant," and "white bud." What suggested the name "white bud" is hard to see, for there is certainly no symptom of the disease which could suggest the term "white." The disease apparently occurs also in other parts of the South. It was found in a shipment of Klondike plants received by the Louisiana Experiment Station from a North Carolina nursery in the fall of 1927.

That the disease has been noticed in other parts of the South is shown by a letter received from Dr. Neil E. Stevens, of the Bureau of Plant Industry, in which he states in part: "There is a disease which we have been calling "dwarf" just as you do, common on Klondikes throughout the South, which seems to be transmitted from the mother plant to the offspring. . . . The one difficulty in working with it is that a good many plants which are stunted by drought or some such cause have a close resemblance to this dwarf condition. About a year ago we brought back from North Carolina a number of Klondike plants which we believed to be dwarf, kept them in the greenhouse during the winter, set them out under good cultural conditions in the spring, and now only something like one-third of the plants show any evidence of disease. This one-third, however, remains in the dwarf condition, and all of the daughter plants are infected. . . ."

The dwarf disease bears a certain resemblance to the strawberry yellows, reported from California,¹ especially with respect to the stunted growth of the plant and the reduction in size and crinkling of the leaves. In other respects the two diseases are apparently distinct. Chlorosis of the leaves, which is the chief characteristic of the yellows, is entirely absent in the dwarf. Furthermore, the dwarf is apparently peculiar to the Klondike variety in Louisiana, and this variety was found to be practically immune to the yellows in California.

IMPORTANCE

No accurate estimate of the damage caused by dwarf can be given at this time, but it is believed that the disease is one of the main causes of the marked reduction in yield of strawberry plantings in Louisiana during recent years. In fields in which the owners have recognized the disease and practiced systematic roguing, relatively few dwarfed plants are found. Incidentally, it may be stated here that those growers who are keen and careful enough to rogue and select their plants are the most successful in this section. In fields where little or no roguing has been practiced, 10 or 20 per cent of the plants may be found dwarfed. It is the unanimous opinion of all growers interviewed that dwarfed plants are practically worthless, for they produce very little fruit or none at all, and what is produced is of small size and has practically no market value.

ETIOLOGY

No pathogene of any kind has been found associated with the dwarf disease. Microscopic examinations of fresh and of fixed sections of leaves and

¹Plakidas, A. G. Strawberry "yellows," a degeneration disease of the strawberry. *Phytopath.* 16: 423-426. 1926.

roots of affected plants have failed to show the presence of any organism in the tissue. Furthermore, the disease cannot be considered as caused by any adverse soil conditions, since it is not limited to any particular soil type, or even to any particular spot in the field. Dwarfed plants are found scattered in the field, many growing side by side with healthy ones.

The general symptomatology of dwarf—the stunting of the plant, the crinkling, cupping and deforming of the leaves, and the fact that affected mother plants transmit the disease to their runner offsprings—suggests that dwarf may be a disease of the degeneration or virus type. The limited experimental evidence thus far obtained supports this view.

EXPERIMENTAL RESULTS

Twenty individuals of a strawberry aphid (probably *Aphis forbesi* Weed) taken from leaves of dwarf plants were placed on each of six young Klondike plants in 6-inch pots in the greenhouse. Each plant was covered by an insect-proof cage. Four other plants used as controls were likewise covered by insect-proof cages, but were not infested with aphids. The plants had been set in the pots ten days earlier, so that at the time the experiment was started only two or three leaves in each plant had unfolded. After the aphids had fed on the plants for ten days, they were killed by spraying the infested plants with a weak nicotine sulphate solution. The control plants were also sprayed with the same solution, to insure uniformity of treatment. The experiment was started on November 11, 1927.

Twenty-six days after inoculation, typical dwarf symptoms had developed in the young unfolding leaves of two of the six aphid-infested plants, and a third had doubtful symptoms. The rest of the inoculated plants, as well as all of the controls, were normal. On a subsequent examination, a week later, two additional plants in the inoculated lot had developed dwarf symptoms. One of the control plants had died from drought, while the remaining three were still healthy. In January, two months after the experiment was started, all six of the inoculated plants had developed the dwarf symptoms, while only one of the controls had developed the dwarf symptoms, the other two remaining normal.

The plants used in the experiment were of the lot of Klondikes obtained from North Carolina. In this lot a relatively high percentage of the plants was subsequently found to be infected with dwarf. Although the results obtained are suggestive, in view of the fact that in the inoculated lot every plant became diseased, they cannot be accepted as final, since it is not known how many of the plants used were originally free from infection. The fact that dwarf symptoms developed on one of the control plants indicated that some of the plants were originally infected even though they did not show

any dwarf symptoms at the time the experiment was started. On the other hand, it is highly improbable that all of the six inoculated plants were originally infected. Further experiments are now in progress, using a large number of plants, and it is hoped that more conclusive evidence as to the nature of dwarf may be obtained from them.

Additional evidence supporting the view that dwarf is a disease of the virus type was obtained from cytological studies. In the pericycle region of young roots of dwarf plants, cells are found which show marked signs of degeneration, and which contain from one to many black-staining (with the iron alum haematoxylin stain) amorphous bodies (Fig. 2). These cell

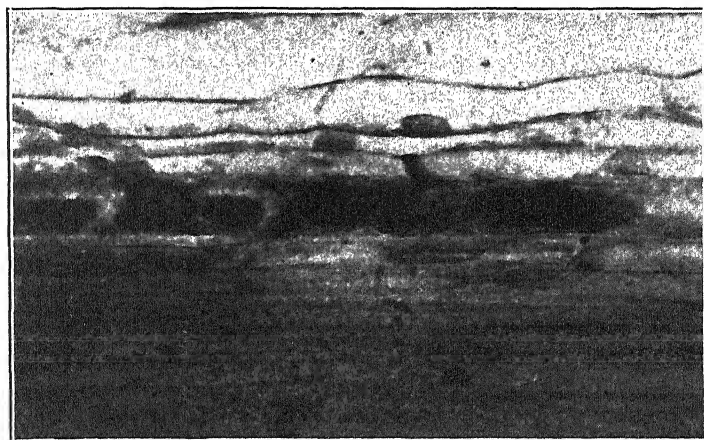


FIG. 2. Photomicrograph of a longitudinal section of a root tip of a dwarf plant showing degeneration of pericycle cells. Note the degenerated nuclei and the black-staining intracellular bodies. Iron-alum haematoxylin stain. Photographed with a Leitz 8× periplane eyepiece and with a Spencer fluorite oil imm. 2 mm. objective. $\times 580$.

inclusions are strikingly similar in appearance and staining reaction to the "X" bodies found by the writer² in the pericycle cells of strawberry roots affected with the yellows disease, and by Rawlins³ in the roots of sugar beets affected with curly-top. The degeneration of these cells is especially noticeable in the case of their nuclei. The nuclei of such cells are almost invariably devoid of nucleoli, and the nuclear membrane appears either to be practically empty, or to contain a small amount of granular, dark-staining material. What may be the nature of this cell degeneration and of the

² Plakidas, A. G. Strawberry xanthosis, a new insect-borne disease. *Jour. Agr. Res.* 35: 1057-1090. 1927.

³ Rawlins, T. E. Cytology of root tips from sugar beets having the curly-top disease (Abst.). *Phytopath.* 16: 761. 1926.

intra-cellular bodies will not be discussed at this time. Further work is being done along this line. Suffice it to state that these abnormalities must have some relation to the disease, since they have not been found in healthy plants.

VARIETAL SUSCEPTIBILITY

No variety susceptibility tests have been carried out, but so far the disease has not been found on any variety except the Klondike. In Louisiana the Klondike is grown almost exclusively. The lot of plants from North Carolina contained, besides the Klondike, about 100 plants each of the varieties Missionary, Big Joe, Lady Thompson, and Hefflins Early; and, while nearly one-third of the Klondike plants proved to be infected, no case of dwarf was found in any of the other four varieties.

This is a preliminary report of a general study of strawberry diseases carried on in this laboratory under the direction of Dr. C. W. Edgerton.

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TRANSMISSION OF POTATO SPINDLE-TUBER BY GRASSHOPPERS (LOCUSTIDAE)¹

R. W. Goss

It has been shown by Schultz and Folsom² that aphids are capable of transmitting the spindle-tuber disease. No reports have been found in the literature showing that other insects have ever been tested. Experiments in Nebraska have confirmed the published results of aphid transmission. However, a careful scrutiny of Nebraska fields during the past seven years has failed to reveal the presence of aphids in large enough numbers to account for all the transmission of spindle-tuber. In experimental plots designed for a study of the natural spread of this disease it was found in three successive years that the disease was transmitted from spindle-tuber to healthy plants in the absence of aphids. Tests were therefore made with other insects commonly found in potato fields.

In 1925, among other tests, 36 inoculations were made using grasshoppers as the transmitting agents. Eight of the inoculated plants became infected with spindle-tuber. The experiments were therefore repeated in 1926, and similar results were obtained.

METHODS

Essentially similar experimental methods were used both in 1925 and 1926. The experimental plot was located at the Scottsbluff substation, Mitchell, Nebr. The potatoes were grown under irrigation. All of the seed potatoes used had been indexed in the greenhouse and were planted in the field in tuber-units. All plants were cut back to a single shoot so that all the tubers in a hill were produced by one stalk.

Grasshoppers were obtained from non-solanaceous plants and were not collected from the vicinity of potato fields. The insects were first tested to determine whether they were already carrying the virus, by placing them on healthy plants either in cages covering the entire plant or in small cages used to cover a single branch. An uncaged plant in the same tuber-unit was used as a healthy control. After one to two days on this plant the grasshoppers were transferred to a caged spindle-tuber plant. They were allowed to feed on this plant two days and then transferred to a healthy plant,

¹ Published with the approval of the Director as Paper No. 46, Journal Series, Nebraska Agricultural Experiment Station.

Acknowledgment is due Mr. R. W. Samson, graduate assistant, who was in charge of the experimental field plots during the summers of 1925 and 1926 and who handled the detailed transmission tests in the field.

² Schultz, E. S., and Donald Folsom. Transmission, variation, and control of certain degeneration diseases of Irish potatoes. *Jour. Agr. Res.* 25: 43-117. 1923.

another uncaged plant of the same tuber-unit being used as a healthy control. In this manner a series of five plants was used for each inoculation.

In addition to these methods, however, an attempt was made to determine the effect of the number of grasshoppers used for an inoculation and the effect of repeated inoculations. The number of grasshoppers used varied from 1 to 10 in the various tests. Single inoculations were made by transferring the insects from a spindle-tuber plant to a healthy plant once, as described above. Double and triple inoculations were made by repeating this operation, the same insects being transferred back to the spindle-tuber plant and again returned to the healthy one. In all the above instances the grasshoppers were allowed to feed upon each plant for two days. The number of insects used in an inoculation was determined by counting only those which were alive and active during the inoculation period. At the end of the inoculation period the grasshoppers were killed with cyanide dust and mounted for identification.

All hills were dug by hand and bagged separately. Records were made of all symptoms appearing in the field and also of all the tuber symptoms. One tuber from each hill was indexed, *i.e.*, one seed piece was planted in the greenhouse under conditions favorable for the appearance of spindle-tuber symptoms. One tuber from a hill has proved sufficient for indexing when only one stalk a hill was allowed in the field, thus decreasing the possibility of partial infection of the hill. In all greenhouse indexing, the plants were grown until tubers were produced and records were made of the symptoms of both tops and tubers. In all inoculation series where positive or doubtful results were secured in the greenhouse, another seed piece from the same tuber as well as another tuber from the same hill was planted in the field the following summer.

If any of the healthy control plants showed symptoms of spindle-tuber in the field, or if the progeny developed symptoms, the entire series was discarded. Only two control plants out of the 140 used in 1925 were infected with spindle-tuber, and all the controls in 1926 remained healthy. The amount of uncontrolled transmission in the field, therefore, can be considered as negligible.

Most of the inoculations were made in late July and early August. The seed was planted about June 1 in both years and harvested about the middle of September.

RESULTS

Current season symptoms appeared on the tops in only a few instances. Symptoms were usually lacking in the tubers of inoculated plants; only 5 hills in 1926 produced typical spindle tubers out of 29 hills which proved to be infected when the tubers were planted.

By planting the progeny of inoculated plants and their controls it was found that 8 of the 36 plants inoculated with grasshoppers in 1925 had be-

come infected. In 1926 there were 29 plants infected from 64 inoculations. Considering the lack of any infection in the healthy controls in 1926, the percentage of infection (46.8 per cent) resulting from inoculations made with grasshoppers is very positive proof of the part these insects may play in transmitting the disease.

Table 1 shows the effect of the number of grasshoppers used for an inoculation and also the effect of repeated inoculations upon the percentage of infection. With increasing numbers and repeated inoculations the percentage of infection increased, until with triple inoculations infection was obtained in 65 per cent of the plants.

Six different species of *Melanoplus* were used in these tests.³ The in-

TABLE 1.—*Effect of the number of grasshoppers and repeated inoculations with spindle-tuber on the number of infected potato plants in 1926*

Number of grasshoppers	Number of inoculations	Number of infected potato plants	Percentage of infection
Single inoculations			
1	1	0	
2	1	0	
3	2	0	
5	3	1	
6	3	1	
8	1	0	
Total	11	2	18
Double inoculations			
?	1	1	
2	4	1	
3	7	2	
4	5	3	
5	3	0	
6	8	5	
7	2	1	
8	2	1	
9	1	0	
Total	33	14	42
Triple inoculations			
2	3	3	
3	6	2	
4	6	4	
5	3	2	
6	1	1	
7	1	1	
Total	20	13	65
Grand total	64	29	46.8

³ Acknowledgment is made to the Entomology Department, Nebraska College of Agriculture, for assistance in the identification of the grasshoppers.

sects were not identified until after the experiments were completed and the insects killed and mounted. Only a few of the tests were found to have been made with unmixed species. *Melanoplus femur-rubrum*, *M. bivittatus*, *M. plumbeus*, and *M. augustipennis* were found to be able to transmit the disease in collections where an average of four out of six insects were identified from the collection. There is of course the possibility that the one or two grasshoppers which were not saved for identification may have been different species. Mixed collections of some of the above species with *M. parkardii* and *M. differentialis* were also successful in transmitting the disease. There is no reason for supposing that any one of these species would be more successful than the others in transmitting the disease in these experiments.

In the 1926 experiments it was found that, of the 77 plants upon which the grasshoppers were fed before being placed on the spindle-tuber plants, 4 became infected while the controls in the same units remained healthy. These same grasshoppers also transmitted the disease to the healthy plants upon which they were fed after feeding on spindle-tuber plants. The only apparent explanation is that these insects were carrying the virus when they were collected.

DISCUSSION

The successful transfer of the spindle-tuber virus to healthy plants by grasshoppers accounts for some of the previously unexplained spread of the disease, particularly in the irrigated districts of western Nebraska. In checking over previous years' results in experimental plots where the natural spread of the disease was being studied, it was found that the greatest amount of spread occurred in those plots where the records of insect infestation showed the greatest number of grasshoppers to be present.

The amount of positive evidence regarding the transmission of virus diseases of the potato by aphids and the few negative tests reported with other insects has tended to discourage attempts to transmit these diseases by many of the common potato insects. The part which aphids play in transmitting the spindle-tuber disease has probably been overestimated in some of the dry-land sections of the west where aphids are not common. Other experiments which have been conducted by the writer with other potato insects indicate that a number of them may be able to transmit the spindle-tuber disease. The experiments are being repeated with larger numbers.

A few attempts made to transmit mosaic with similar insects have given only negative results. In addition, in the plots being used for the study of the natural spread of potato virus diseases, it was found that both mild mosaic and rugose mosaic failed to spread under the same conditions that resulted in a considerable spread of spindle-tuber.

NEBRASKA AGRICULTURAL EXPERIMENT STATION,
LINCOLN, NEBRASKA.

COPPER SULPHATE AS A REMEDY FOR EXANTHEMA IN PRUNES, APPLES, PEARS, AND OLIVES

RALPH E. SMITH AND HAROLD E. THOMAS

The disease called "exanthema" or "dieback" in citrus trees has received considerable attention both in Florida and California. It is a trouble marked by very characteristic symptoms, as specific as those of any plant disease, parasitic or "physiological." So far as we are aware, exanthema has never been described on any but citrus trees, particularly the orange and grape fruit. For a long time, however, it has been known that in Cali-

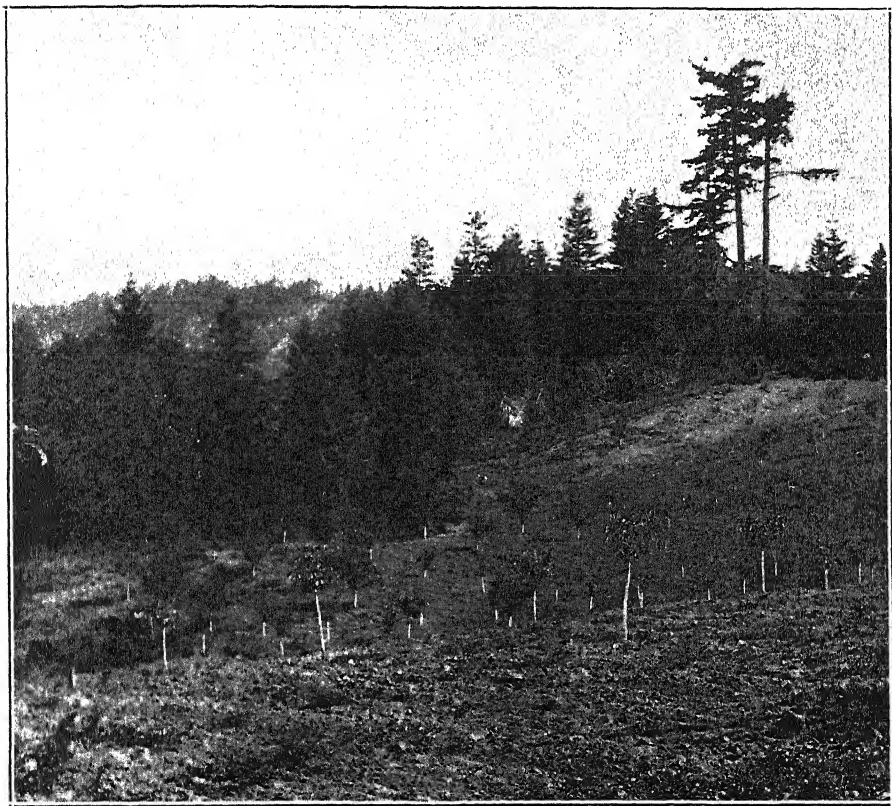


FIG. 1. Four-year-old French prune trees with exanthema.

ifornia a similar disease is not uncommon on fruit trees of various species, usually in spots of shallow soil where drainage is poor and the soil moisture varies between extremes of saturation and drouth. Defects of this sort are not obvious in every instance, however.

The recently discovered fact that application of copper sulphate to the soil has a strikingly remedial effect in some of these cases, as has long been known to be true with citrus exanthema, adds weight to the conclusion that the diseases are related and revives the interesting question as to the actual cause of this phenomenon.¹ A few cases may be described.

EXANTHEMA IN FRENCH PRUNES

A typical case occurred in a young orchard of French prune trees (*Prunus domestica*), planted on supposedly good soil which had originally supported a particularly heavy growth of the coast redwood or "big tree" of California (*Sequoia sempervirens* (Lamb.) Endl.) and other native trees (Fig. 1). This was in a region of heavy winter rainfall (50–100 inches) with no rain in summer (May–November) and no irrigation. Considerable variation exists in the physical structure, moisture condition, and topography of the soil in various parts of the orchard, so that it seems impossible to connect the disease with any of these factors.

Symptoms.—Most of the trees in this orchard have made very little growth, being not much larger after four or five years than when first planted (Fig. 2). Each spring vigorous new shoots start out, but in June the terminal buds wither and fall out and the terminal leaves turn a yellowish color. Often lateral shoots push out and then die back in the same manner. Eruptions of the bark and swollen and multiple buds occur, just as in the citrus disease (Fig. 3). The disease is not confined to prunes but shows itself on the same ranch to a greater or less extent on apples, pears, and Japanese plums. It also occurs on other ranches in the same vicinity.

During the spring of 1926 the following fertilizing materials were applied to rows of 16 trees each:

Row 3, ammonium sulphate 1 lb. a tree, sodium nitrate 1 lb., potassium sulphate 2 lbs., March 26.

Row 5, superphosphate 5 lbs. a tree, potassium sulphate 2 lbs., March 26.

Row 7, ammonium sulphate 1 lb. a tree, sodium nitrate 1 lb., superphosphate 5 lbs., March 26.

Row 9, ammonium sulphate 1 lb. a tree, sodium nitrate 1 lb., superphosphate 5 lbs., potassium sulphate 2 lbs., March 26.

¹ Of interest in this connection is the effect of copper sulphate on plant growth in peat lands. See Allison, R. V. et al. Stimulation of plant response on the raw peat soils of the Florida Everglades through the use of copper sulphate and other chemicals. Fla. Agr. Exp. Sta. Bul. 190: 35–80. 1927.



FIG. 2. Closer view of French prune trees with exanthema.

Row 11, superphosphate 5 lbs. a tree, Feb. 27.

Row 13, ammonium sulphate 2 lbs. a tree, Feb. 27.

Row 15, dried blood 4 lbs. a tree, Feb. 27.

Row 17, ammonium sulphate 2 lbs. a tree, superphosphate 5 lbs. a tree, Feb. 27.

Row 19, poultry manure 100 lbs. a tree, March 15.

Row 21, nitrate of soda 1 lb. a tree, March 26, and 1 lb. May 9.

Row 23, calcium nitrate 1 lb. a tree, March 26, and 1 lb., May 9.

Row 24, potassium sulphate 2 lbs. a tree, March 26.

In addition to this, four trees received an application of five pounds each of commercial copper sulphate on March 27. In each case the material was scattered about the tree and dug into the soil by hand.

During the season of 1926 no effect whatsoever could be seen in any of the trees except in the case of the four which received the copper sulphate. On these a vigorous, normal growth developed, with green foliage and ter-



FIG. 3. French prune twigs affected with exanthema; showing dead, swollen and multiple buds and exanthema of bark.

minal buds remaining throughout the season. The old bark lesions healed over, and the trunks and limbs became smooth and healthy looking. These four trees were outstanding in the orchard for their thrifty growth and appearance.

In the spring of 1927 four trees were treated with an application of $2\frac{1}{2}$ lbs. each of c. p. copper sulphate, and ninety-six trees with $2\frac{1}{2}$ lbs. each of commercial bluestone. Ten other trees received 25 lbs. each of double superphosphate, three trees 50 lbs. each of the same, and three trees 500 cc. each of commercial sulphuric acid, diluted with water to 5,000 cc. Again nothing showed the slightest effect except the copper sulphate. All the trees thus treated, except one or two which were nearly dead from the disease, responded markedly in growth, color, and healing of bark lesions. In August, 1927, measurements were made of the growth of leading shoots of many of the treated and untreated trees. On eight representative trees of those which had been treated with copper sulphate, 57 leading shoots averaged 37 inches growth in length in 1927. The maximum was 76 inches, and there were 14 shoots more than 50 inches long and 51 over 20 inches. On the nine best untreated trees adjacent to the others, the best 68 shoots averaged 13 inches growth in 1927. The maximum was 29 inches, and there were nine over 20 inches. At this time the treated trees were of a green, healthy color, with normal terminal buds. The untreated were chlorotic and sickly-looking and had lost practically all their terminal buds.

The four trees which were treated with copper sulphate in 1926 received no further applications but continued to grow normally and vigorously in 1927. Figure 4 shows two of these trees in August, 1927, together with some of the surrounding, untreated trees. The treated ones were rather worse than these before the application of copper sulphate.

EXANTHEMA IN APPLES

A very badly affected six-year-old apple tree stood in a garden on the same ranch. The whole top of this tree was a mass of brushy, yellow, die-back shoots. On June 23 a trench a foot deep was dug all around this tree, and two pounds of copper sulphate crystals sprinkled in the trench half way around the circumference of the tree. The trench was then filled with water, after separating the copper sulphate portion from the other half with earth. On August 17 a much improved condition was apparent all over the top of the tree on that side which had received the copper sulphate. On the untreated side the twigs were in even worse condition than before.

EXANTHEMA ON PEAR TREES

In another unirrigated region in California many Bartlett pear trees have typical symptoms of exanthema. Copper sulphate applied to the soil

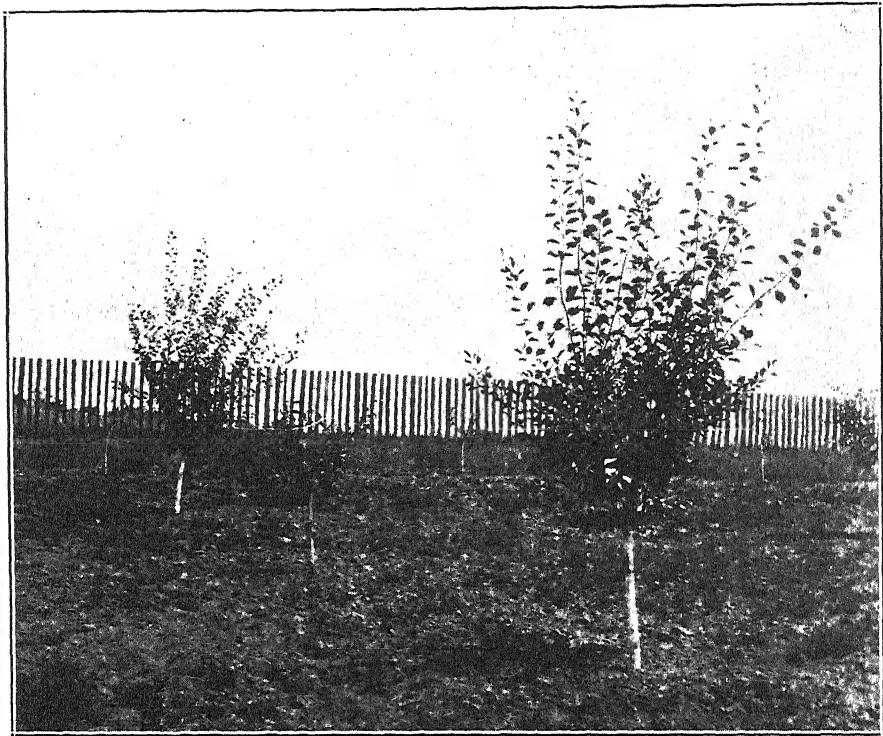


FIG. 4. Effect of copper sulphate on exanthema of French prune trees. Five pounds a tree was applied to alternate trees the previous year.

about some of these trees in the spring of 1927 brought about as decisive an improvement as that in the case of the prune and apple trees.

EXANTHEMA IN OLIVES

In still another district, typical exanthema symptoms occur quite abundantly on young olive trees. The terminal buds and shoots wither and die, laterals push out and meet the same fate, and eruptions develop in the bark. In this case the trouble is mostly confined to spots in the orchard which become very wet during rainy weather and irrigation, and very dry and hard between times. Trial of copper sulphate was suggested in this case, and growers have reported beneficial results.

Investigations on the soils of these different localities, which appear to vary widely from one another in many respects, both physical and chemical, are being conducted by the division of plant nutrition.

DIVISION OF PLANT PATHOLOGY,
COLLEGE OF AGRICULTURE,
UNIVERSITY OF CALIFORNIA.

CLASSIFICATION OF COPPER FUNGICIDES¹

E. B. HOLLAND AND G. M. GILLIGAN

In considering the numerous copper fungicides that have been recommended by different investigators, or introduced by various manufacturers, sufficient diversity will be observed to warrant a classification, first, based on the mode of application, into (A) sprays and (B) dusts; and secondly, based on the solubility of the product at the time of application, into (I) soluble and (II) insoluble. Such a classification is necessarily more or less arbitrary and lacks scientific precision, but has proved extremely serviceable.

With sprays, the vehicle is water and the soluble forms of copper are true solutions of simple salts or cuprammonium compounds and the insoluble forms are colloids (suspensoids or emulsoids) or suspensions (fine or coarse). The soluble forms of copper are immediately active, toxic to foliage, especially under slow drying conditions, easily distributed, and the residue on evaporation inconspicuous and poorly adhesive. The adhesiveness to the foliage depends mostly on the chemical composition. A deposit of copper sulphate remains soluble and is readily removed by rain. The cuprammonium compounds are probably rendered insoluble more rapidly than the acetates, but the latter, particularly the basic, are more persistent on the leaves. Occasionally substances are added to Bordeaux or Burgundy mixtures with a view of producing soluble derivatives of copper and thereby increasing the activity of the mixture. Of these, sugars and some hydroxy-organic acids are the most promising, although the practice is not recommended.

The insoluble forms of copper as a class are slowly active, comparatively non-toxic to the plant, more difficult to apply, and the residue on evaporation readily visible and adhesive. The adherence depends largely on the physical characteristics, and the colloids and gelatinous products are considered the most efficient. The insoluble forms of copper employed are generally basic sulphates or basic carbonates, either home-made or commercial. Cupric hydroxide and so-called colloidal copper have recently been offered for trial by the producers. The efficiency of all insoluble deposits of copper, whether natural or air-converted, depends on a small amount being rendered soluble by atmospheric agents, osmosis or other factors

¹ From the Department of Chemistry, Massachusetts Agricultural Experiment Station. Printed with the permission of the Director of the Station.

The writers wish to acknowledge the assistance of Dr. Paul Serex, of the Department of Chemistry of the Massachusetts Agricultural College.

acting singly or jointly. Substances are often added with a view of increasing the degree of suspension, assuring more uniform distribution (spreading) and increasing adhesiveness. As the addition of such materials generally tends to lower the availability of the copper by reducing the concentration of the residue, or by forming less ionizable derivatives, only a small amount of such substances (0.10 per cent of the spray or less) should be employed.

With dusts the vehicle may be a relatively inert carrier such as talc, steatite, kaolin, clay, Fuller's earth, infusorial earth, pumice, gypsum, coal dust, sulphur, aluminum hydroxide, or a more reacting (protective) carrier such as calcium carbonate or calcium hydroxide. The soluble forms of copper may be saturated solutions or pulverized, partially dehydrated or anhydrous salts, generally the sulphate, incorporated with an inert vehicle. With a reacting vehicle in the presence of moisture, a portion of the copper at least would be rendered insoluble. This sub-group might be omitted on the grounds that most of the copper present is either soluble or insoluble on application, but, as some of the mixtures occupy an intermediate position, it is retained. The insoluble forms are copper stearate and those mentioned under sprays.

The following tentative classification is presented:

Sprays.

I. Soluble—True solutions.

(a) Simple salts.

(1) Copper sulphate $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.

(2) Normal copper acetate (verdet neutre) $(\text{CH}_3\text{COO})_2\text{Cu} \cdot \text{H}_2\text{O}$.

(3) Basic copper acetate² (verdet gris) $(\text{CH}_3\text{COO})_2\text{Cu} \cdot \text{CuO} \cdot 6\text{H}_2\text{O}$.

(b) Cuprammoniums.

1. Ammonia (hydroxide) soluble.

(1) Copper sulphate (eau celeste).

(2) Cuprammonium sulphate $\text{Cu}(\text{NH}_3)_4\text{SO}_4 \cdot \text{H}_2\text{O}$.

(3) Burgundy mixture (modified eau celeste).

(4) Malachite (cupram).

2. Ammonium carbonate or bicarbonate soluble.

(1) Copper sulphate (Johnson's mixture).

(2) Cuprammonium sulphate.

(3) Burgundy mixture.

(4) Malachite.

(c) Organic copper derivatives in alkaline mixtures (Bordeaux, Burgundy) produced by:

(1) Sugars, dextrin, glycerol, etc.

² The basic portion is probably a suspensoid.

- (2) Hydroxy-organic acids.
- (3) Biuret compounds (probably colloidal).

II. Insoluble.

- (a) Colloids.
 - 1. Suspensoids.
 - (1) Colloidal copper.
 - 2. Emulsoids.
 - (1) Pickering spray emulsion.
 - (2) Bordo emulsion.
 - (3) Cuprammonium sulphate emulsion (Kupfer preparat Gmund).
- (b) Suspensions.
 - 1. Fine (gelatinous, often termed colloidal).
 - (1) Bordeaux mixture.
 - Highly basic, basic and alkaline.
 - Neutral.
 - Acid (flocculent precipitate).
 - (2) Pickering sprays.
 - Lime water precipitated.
 - Barium water precipitated.
 - (3) Burgundy mixture (soda Bordeaux).
 - (4) Sodium bicarbonate Bordeaux.³
 - (5) Mixed carbonates Bordeaux.³
 - (6) Bordeaux paste.
 - (7) Colloidal copper (proprietary).
 - 2. Coarse.
 - (1) Dry Bordeaux (Strawsonite).
 - (2) Basic copper sulphate $\text{CuSO}_4 \cdot 3\text{CuO} \cdot 4\text{H}_2\text{O}$.
 - (3) Copper hydroxide.

B. Dusts.

I. Soluble.

- (a) With inert vehicles.
 - (1) Sulphosteatite (Fostite).
 - (2) Various French and German mixtures (copper sulphate, etc.).
 - (3) Sulphated sulphur (Blight powder).
- (b) With more or less reacting vehicles.
 - (1) Sulfatine.
 - (2) Several foreign mixtures (copper sulphate, lime, etc.).
 - (3) Monohydrated or anhydrous copper sulphate and hydrated lime.

II. Insoluble.

- (1) Dry Bordeaux.
 - David powder, Podechard powder, Cuprosteatite, etc.
- (2) Basic copper sulphate $\text{CuSO}_4 \cdot 3\text{CuO} \cdot 4\text{H}_2\text{O}$.
- (3) Malachite (disinfectant) $\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$.
- (4) Copper stearate $(\text{C}_{17}\text{H}_{35}\text{COO})_2\text{Cu}$.

³ Contains more or less soluble copper.

It is to be regretted that all of the products cannot be listed as chemical entities, but this is impossible from present knowledge. Furthermore, the lack of control measures in the preparation or manufacture of the different products makes it impossible to assure always a single active compound, and often there is produced a mixture together with an excess of precipitant and of more or less inactive by-products. The principal objective is to obtain an active product of good physical characteristics suitable for application as a spray or as a dust. Any classification based on one or two characteristics must necessarily show inconsistencies in other respects unless the subdivisions are increased to a point unwarranted in this connection. Innumerable other compounds, preparations, and modifications of sprays and dusts have been mentioned in literature, but, seemingly at least, are not of sufficient importance to be listed.

DEPARTMENT OF CHEMISTRY,

MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION,

AMHERST, MASS.

PHYTOPATHOLOGICAL NOTES

The transmission of potato black-leg by the seedcorn maggot in Maine. Proof by Leach that the seedcorn maggot (*Hylemyia cilicrura* Rond.) disseminates potato black-leg in Minnesota¹ was followed by observations on this phenomenon in Aroostook County, Maine.

Flies of this species were collected in abundance in 1925, 1926, and 1927. In 1927 a very severe black-leg epiphytotic occurred. Many unexplained cases of high black-leg percentage were observed in fields planted with seed potatoes thought to have been disease-free. On June 20 a collection of the adult flies was made in a fallow field. Some of them were kindly identified by Dr. O. A. Johannsen of Cornell University. The rest were placed in 8 covered glass jars containing sterilized soil and healthy seed pieces from fungicide-treated tubers. Each jar contained 5 seed pieces, partly covered by the soil, and from 3 to 5 of the introduced flies. The seed tubers were all represented by seed pieces in 2 similar jars without flies, these serving as controls. About 4 weeks later a rapid seed-piece rot developed in 3 of the jars containing insects. In these 3 jars numerous maggots were burrowing into the rotted seed pieces. The jars were kept in the light and the soil was kept watered. Of the 15 seed pieces contained in the 3 jars infected with maggots, 5 were destroyed before producing plants, while the other 10 produced 10 black-leg plants in the jars. The flies failed to produce maggots in 5 jars, the cause not being understood. The seed pieces in these 5 jars and in the 2 control jars remained healthy and produced healthy plants.

Some of the maggots were placed in proximity to other freshly cut potato seed pieces. The maggots soon attacked this potato material through the cut surfaces and burrowed deeply, initiating a rapid soft rot like that caused in the preceding experiment.

Field observations revealed that the same type of maggots were common in the soil beneath black-leg plants. Collections of these maggots were also capable of causing a rapid rot in freshly cut potato tubers.

From these results it is concluded that the seedcorn maggot is a factor to be considered in the control of potato black-leg in Maine.—REINER BONDE, Agricultural Experiment Station, Orono, Maine.

¹ Leach, J. G. The seed-corn maggot and potato blackleg. *Science* 61: 120. 1925.

———. The relation of the seed-corn maggot (*Phorbia fusciceps* Zett.) to the spread and development of potato blackleg in Minnesota. *Phytopath.* 16: 149-175. 1926.

Bacterium stizolobii (Wolf) comb. nov. syn. *Aplanobacter stizolobii*
In the original publication on this plant pathogen (Phytopath. 10: 73-80. 1920) it is described as non-motile and Morrey's modification of Loeffler's method failed to demonstrate any flagella. In a later study by the writer a single, short, polar flagellum was stained both by the Casares-Gil and by David Ellis methods. The organism is apparently non-motile in most cultures, also when examined directly from leaf lesions. Slight but definite motility was observed in some young, beef-agar cultures, and from these the flagella were demonstrated.—LUCIA McCULLOCH, Department of Agriculture, Washington, D. C.

Bacterium maculicola (McC.) nom. emend. syn. *Bacterium maculicolum*. Original publication: A spot disease of cauliflower, U. S. Dept. Agr., Bur. Plant Indus., Bul. 225. 1911. The ending *a* is the preferred spelling.—LUCIA McCULLOCH, Department of Agriculture, Washington, D. C.

A question of correct usage. It is quite the usual thing for beginners in biologic science to find Latin plurals a stumbling block, but it seems that many experienced workers are tripping over the same stone in their published scientific contributions. I refer to the frequent misuse of the Latin plural form, especially the plural ending in *-a*, in forming compound words where ordinary English usage calls for the singular. To illustrate: When we refer to fungous hyphae which bear spores we properly speak of "spore-bearing" hyphae. When we wish to refer to hyphae which bear conidia the proper expression is "conidium-bearing," but frequently the combination "conidia-bearing" is used instead. In the same way "bacteria-like" is used where "bacterium-like" ought to be employed. The absurdity of these and other similar abnormal combinations is perfectly apparent if we listen to the odd sound of their approximate equivalents, e.g.—spores-bearing, germs-like.—H. P. BARSS, Oregon State Agricultural College, Corvallis, Oregon.

Notice of field meeting of the Southern Section of the American Phytopathological Society. At the Nashville meeting of the American Phytopathological Society the members of the Southern Section voted unanimously to hold a field meeting at a time and place to be decided upon later. Accordingly arrangements have been made for a three-day field meeting to be held in Georgia, July 11-13, 1928, and a cordial invitation to attend is extended to all who are interested in diseases of southern crops. The Georgia plant pathologists have planned an excellent program with a num-

ber of unique and valuable features, and it is hoped that a large number will plan to attend.

Briefly, the itinerary outlined is as follows:

July 11. Assemble at Experiment, Georgia, at 10 A. M. See the research work of the Experiment Station, including results of cotton seed treatments, work on pepper diseases, tomato wilt, peach rosette, peanut diseases, and other plant diseases. Attend the dedication of the new library and laboratory building of the Experiment Station and barbecue at noon. 8 P. M. Discussion of the day's observations and plans for the following day.

July 12. Drive 64 miles to Fort Valley. See the work of the United States Peach Insect and Disease Laboratory on peach insects, brown rot, bacterial spot, and "Phony" peach. Visit some of the commercial orchards and packing houses. Drive 80 miles to Albany, and spend the night there. 8 P. M., assemble for discussion, etc.

July 13. See airplane dusting of pecan grove. See some of the principal pecan diseases and control methods as practiced in commercial groves about Albany. Drive 45 miles to the Coastal Plain Experiment Station, Tifton, to see the work on cantaloupe and tobacco diseases. Drive 50 miles to Thomasville to see the work on pecan and other crop plant diseases being done at the United States Pecan Disease Laboratory and at the Field Laboratory of the State Board of Entomology.

The trip will end officially at Thomasville, Georgia, but arrangements have been made for those interested in tobacco diseases to visit the Florida Tobacco Station, Quincy, Florida.

Automobile transportation will be furnished locally for visitors who do not find it convenient to bring their own cars. Those planning to attend are asked to communicate as soon as possible with Dr. B. B. Higgins, Agricultural Experiment Station, Experiment, Georgia, indicating whether or not they desire transportation furnished for the field trip.—V. H. YOUNG, Secretary, Southern Section, American Phytopathological Society.

REPORT OF THE NINETEENTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

ADDITIONS AND CORRECTIONS TO REPORT OF EIGHTEENTH ANNUAL MEETING

1. The following paragraph was inadvertently omitted from the Secretary's report of the Philadelphia meeting (*Phytopath.* 17: 343-353. 1927.)

The matter of a permanent endowment for PHYTOPATHOLOGY was proposed by E. C. Stakman. It was pointed out that the journal has practically reached the limitations made possible by its present income and that more money is needed for expansion. It was voted to have a committee on permanent endowment and the newly elected President later appointed E. C. Stakman, C. R. Orton and I. E. Melhus. The fund was started by a twenty-dollar check from H. H. Whetzel.

2. In the report of the Business Manager of PHYTOPATHOLOGY the statement of liabilities (page 347) should read "Outstanding checks—\$914.98" instead of "none" as printed.

THE NASHVILLE MEETING

The joint annual meeting of the American Phytopathological Society and its Southern Division was held at Nashville, Tennessee, December 28-30, 1927, in conjunction with the American Association for the Advancement of Science and affiliated societies. The hotel headquarters were at the Sam Davis Hotel and the meeting rooms were in the Demonstration School of the George Peabody College for Teachers. About 150 members were in attendance.

The program was made up of 90 papers, 18 in general sessions, 4 in joint session with Section G, 12 in joint session with the Mycological Section of the Botanical Society of America, and the remainder in sessions for papers on tobacco diseases (8), diseases of southern crops (7), cereal and forage crop diseases (11), potato and vegetable diseases (11), fruit diseases (9), diseases of ornamental plants (7), and diseases of sweet potatoes (3). Of the 90 papers, 82 were contributed by this Society, 4 by the Mycological Section, and 4 by Section G.

At a conference on market pathology and its relation to extension activities, talks were given on various phases of the work by F. C. Meier, Chairman; William Turner, Horticultural Agent of the Central of Georgia Railroad; F. G. Robb, of the U. S. Bureau of Agricultural Economics; J. I. Lauritzen and R. J. Haskell, of the U. S. Bureau of Plant Industry; V. H. Young, of the Arkansas Agricultural Experiment Station; J. J. Taubenhaus, of the Texas A. & M. College, and others.

At the Plant Disease Survey supper and round table, the recent surveys in Missouri, Iowa and Utah were discussed.

The Phytopathologists' dinner was held Thursday evening, December 29, at the Nashville Chamber of Commerce. President Barrus, being located in Porto Rico for a year, found it impossible to be in attendance. Therefore Vice-President Barss, of Oregon, officiated both at the dinner and at the other sessions of the Society. Dr. L. R. Hesler was in charge of the arrangements for the after-dinner program which, guided by our efficient and effective Vice-President, proved very entertaining. A quartet of real southern negro singers, an interesting letter from President Barrus read by Dr. Mel T.

Cook, an impersonation of the ghost of Charles Darwin, by F. D. Fromme, a talk by Watson Davis of Science Service, and the reading of, and awarding of prizes for phytopathological limericks by Drs. E. C. Stakman and L. R. Jones made up the program.

OFFICERS AND REPRESENTATIVES

The following officers were chosen:

President, H. P. Barss, Oregon Agricultural College, Corvallis, Ore.

Vice-President, F. D. Heald, Agricultural Experiment Station, Pullman, Wash.

Councilor (two years), F. D. Fromme, Virginia Polytechnic Institute, Blacksburg, Va.

Editor-in-Chief of Phytopathology (three years), E. C. Stakman, University of Minnesota, University Farm, St. Paul, Minn.

Editors (three years), H. M. Quanjer, Editor for Europe, Institut voor Phytopathologie, Wageningen, Holland; L. R. Hesler, University of Tennessee, Knoxville, Tenn.; M. N. Levine, University of Minnesota, St. Paul, Minn.

Associate Editors (three years), W. B. Tisdale, Tobacco Experiment Station, Quincy, Fla.; S. M. Zeller, Oregon Agricultural College, Corvallis, Ore.; Margaret Newton, Manitoba Agricultural College, Winnipeg, Canada; C. W. Bennett, Michigan Agricultural College, East Lansing, Mich.; Donald Folsom, Maine Agricultural Experiment Station, Orono.

Business Manager (one year), R. J. Haskell, Bureau of Plant Industry, Washington, D. C.

Advertising Manager (one year), J. F. Adams, Agricultural Experiment Station, Newark, Del.

Representatives on the Council of the American Association for the Advancement of Science (one year), Donald Reddick, New York State College of Agriculture, Ithaca, N. Y., and C. W. Edgerton, Louisiana Agricultural Experiment Station, Baton Rouge, La.

Members of the Advisory Board (three years), J. A. Stevenson, Bureau of Plant Industry, Washington, D. C., to succeed F. C. Meier as Commissioner for the U. S. Department of Agriculture; F. L. Drayton, Dominion Department of Agriculture, Ottawa, Canada, to succeed J. E. Howitt as Commissioner for Canada; and I. E. Melhus, Iowa State College of Agriculture, Ames, Iowa, to succeed M. F. Barrus as Commissioner at large.

Members of the Board of Governors of the Crop Protection Institute (three years), J. F. Adams, Agricultural Experiment Station, Newark, Del., to succeed N. J. Giddings. The other two members of the board are I. E. Melhus and H. W. Anderson.

Representative (Elector) on the Division of Biology and Agriculture of the National Research Council (three years), F. D. Fromme, Virginia Polytechnic Institute, Blacksburg, Va.

Member of the Permanent Committee on Necrology, M. B. Waite, Bureau of Plant Industry, Washington, D. C., to succeed Haven Metcalf, resigned. The other members of this committee are G. P. Clinton and L. R. Jones.

The following temporary committees were appointed by the President to serve throughout the meetings: *Resolutions Committee*, J. C. Walker, A. G. Johnson and C. R. Orton; *Auditing Committee*, W. H. Tisdale and B. F. Dana; *Committee on Publicity*, F. J. Schneiderhan, J. F. Adams, and G. H. Coons; *Committee on Elections*, L. M. Massey, R. S. Kirby and M. W. Gardner.

The Southern Division of the Society chose the following officers for 1928 at its business session, December 29. President, B. B. Higgins; Secretary, V. H. Young; Councilor, W. B. Tisdale.

REPORT OF THE SECRETARY TREASURER, 1927

At the close of the Philadelphia meeting the membership totaled 716. During the year a loss of 31 members was sustained—22 by suspension for non-payment of dues, 7

STATEMENT OF ACCOUNTS FOR 1927, AS OF DECEMBER 19, 1927

Receipts:

Balance from 1926		\$2,129.06
Annual dues: 1924	\$ 4.00	
1925	14.00	
1926	32.00	
1927	1,075.70	
1928	1,719.21	
1929	13.50	
	<hr/>	2,858.41
Excess dues		1.49
Interest on checking account		19.24
Interest on time deposit		30.00
Cash returned by Secretary-Treasurer, expenses of meeting		9.17
Dues in Physiological Section received with annual dues		1.50
Sales received with annual dues		5.62
	<hr/>	\$5,054.49

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY	\$1,962.00	
Secretarial work	295.75	
Postage stamps	39.00	
Telegrams	2.47	
Printing (preliminary announcements, dinner tickets, programs, application blanks, ballots, nomination ballots, envelopes)	169.50	
Expense of lantern slides of seals of Society	5.55	
Expenses of Secretary-Treasurer at annual meeting	75.00	
Work on Erwin F. Smith testimonial	36.50	
Miscellaneous expenses (account cards)94	
Dues in Physiological Section paid	1.50	
Checks returned by bank	12.00	
Amount transferred to Sinking Fund for investment	498.00	
	<hr/>	\$3,098.21
Balance on checking account		\$1,956.28
Outstanding check		43.50
		<hr/>
		\$1,999.78
Amount of above receipts credited to 1928-1929	\$1,732.71	
Amount due Sinking Fund	24.00	
Sales due PHYTOPATHOLOGY	5.12	
	<hr/>	\$1,761.83
Actual balance for 1927		\$ 237.95

by resignation, and 2 by death, making the total membership 685 at the close of 1927. Of these, 74 were life members paid in full, 39 were life-sustaining members paying currently, and 572 were annual regular members. At Nashville 65 new members were elected, thus bringing the total up to 750, a net gain of 34.

In the United States, New York and Minnesota tied for first place in the securing of new members with 7 each. Wisconsin and Iowa tied for second place with 5 each. These were followed by Florida with 3, California and Delaware with 2, and 18 other states with one each. Of the foreign countries Japan led with 6 new members, then Canada with 4, England and Australia with 2 each, and Czechoslovakia and India with one each.

The increase of members in foreign countries during the recent years is notable. In 1924, 8 foreign members were elected, in 1925—9, in 1926—19 and in 1927—17.

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1927

Since June, 1924, PHYTOPATHOLOGY has been printed by the Science Press Printing Company, of Lancaster, Pa., and it is generally agreed that a very creditable piece of work has been done. It is with deep regret that we report the death on February 3, 1927, of the Manager of the Company, Mr. A. E. Urban, who took a great personal interest in the welfare of the journal. A printer by trade and a botanist at heart, he was especially fitted to handle and to appreciate our work. Since his death the business has been efficiently carried on by Mr. Jaques Cattell, Secretary of the Company, and his associates.

The close of the year finds PHYTOPATHOLOGY not quite so well off financially as in other recent years, the bank balance being \$585.08 as compared with \$1,103.84 last year. This seems to be due principally to decreased receipts from sales of back numbers and advertising. In addition to the balance of \$585.08 and exclusive of the sinking fund the journal has \$1,000 invested in securities.

The number of subscribers in good standing at the close of the year is 420, of which 252 are foreign and 168 domestic. The present edition of PHYTOPATHOLOGY is 1,525 copies, of which 1,136 are being mailed out.

The sinking fund now amounts to \$5,274.00.

STATEMENT OF ACCOUNTS FOR 1927, AS OF DECEMBER 19, 1927

Receipts:

Balance from 1926	\$1,103.84	
Subscriptions (\$686.40 for 1928, \$2.50 for 1929)	2,483.97	
Sales, PHYTOPATHOLOGY	370.20	
Phytopathological Classics	112.18	
Advertising: 1926	\$146.08	
1927	719.53	865.61
Interest on Sinking Fund and investment		392.32
Donation to PHYTOPATHOLOGY Endowment Fund		20.00
Amount due Sinking Fund from Society		498.00
Member subscriptions for 1927		1,962.00
Sinking Fund investment paid up (reinvested)		500.00
		<hr/>
		\$8,308.21

Expenditures:

Manufacturing PHYTOPATHOLOGY:

Vol. XVI, No. 12	\$ 635.65	
Vol. XVI, Index	138.66	
	<hr/>	\$ 774.31
Vol. XVII, No. 1	382.71	
Vol. XVII, No. 2	450.63	
Vol. XVII, No. 3	430.14	
Vol. XVII, No. 4	366.09	
Vol. XVII, No. 5	589.42	
Vol. XVII, No. 6	391.44	
Vol. XVII, No. 7	553.71	
Vol. XVII, No. 8	360.95	
Vol. XVII, No. 9	570.63	
Vol. XVII, No. 10	572.05	
Engravings for Vol. XVII	796.09	
	<hr/>	\$5,463.86
		<hr/>
Manufacturing Phytopathological Abstracts		\$6,238.17
Manufacturing Phytopathological Classics		26.80
Expenses of Editor-in-Chief (editing manuscripts, etc.)		232.90
Expenses of Advertising Manager (postage, stamped envelopes, stenography)		65.62
Postage, Business Manager		43.62
Miscellaneous expenses (account books, expressage)		15.00
Secretarial work		2.10
Sinking Fund invested and reinvested with accrued interest.....		91.50
Purchase copy of PHYTOPATHOLOGY		\$1,006.42
		1.00
		<hr/>
		\$7,723.13
		<hr/>
Balance		\$ 585.08
Outstanding checks		49.64
		<hr/>
		\$ 634.72

Assets:

Cash in bank (less outstanding checks)	\$ 585.08
Sinking Fund (invested)	5,250.00
Amount due Sinking Fund (now in Society's account)	24.00
First mortgage (surplus)	1,000.00
Amount due from subscriptions	826.50
Amount due from advertising	202.40
Amount due from sales made	20.22
Due from Society for sales included with dues	5.12
Checks on hand to be deposited	140.15
	<hr/>
	\$8,053.47

Liabilities:

None.

REPORT OF THE EDITOR-IN-CHIEF OF PHYTOPATHOLOGY

Volume 17 of PHYTOPATHOLOGY contains 836 pages, comprising 69 articles, 15 notes, 3 reports, and 3 book reviews; 31 plates and 169 text figures. Ninety-eight manuscripts were submitted, of which 22 were returned for revision and 4 rejected. The papers published in Volume 17 have been, on the average, shorter than those published in 1926. Fewer papers were rejected, although a considerable number had to be returned to the authors for major revision or for approval of changes made by the editorial staff. Volume 17 contains a larger number of illustrations than the previous volume, which will probably mean a greater expense to the Society.

In our report last year it was stated that five months, the average length of time required to get a paper into print, probably could not be shortened very much. During 1927, however, we have been able to publish manuscripts, on the average, in four months from the date of receipt. A few have required six months; a few have been published in two months; the number which required five months is balanced by those published in three months; and the larger number by far have appeared in four months. We have accomplished this by putting into each number most of the galleys on hand. This procedure has several disadvantages: it puts a burden on the printer by requiring him to print material as soon as it is received; we lead a somewhat hand-to-mouth existence by enriching one number and impoverishing the next; and on both of these accounts numbers are likely to be behind schedule and unequal in size. Only by doing this, however—by using all of the galleys available when a number is being made up—has it been possible to shorten the time required for publication.

Acknowledgement is due each of the associate editors for indexing one number of Volume 17. Some of the associate editors, in addition, have rendered very valuable service in approving or rejecting manuscripts submitted to them for transmittal to the editor-in-chief. For this assistance the editor-in-chief is very grateful.

According to the editorial committee for Phytopathological Abstracts, the papers submitted for the program of the annual meeting were a great deal too long, in many cases, to come within the 200-word limit. The editorial committee had insufficient time to return the abstracts to authors; neither could they take the time, nor the risk, to condense papers enough to make them come within the word limit. Some of the abstracts were over 300 words in length. There seems to be little excuse for this. The committee therefore recommended that the procedure for next year be changed: the abstracts should be requested earlier, say November 1; the editorial committee would return those which did not come within the word limit, and those which do not offer worth-while information. By moving the date of receipt forward, there would be time to edit the abstracts carefully, and time to satisfy the committee and the authors in regard to points of conflict.

Detailed editing of all manuscripts has been done by Helen Hart and Laura M. Hamilton. They wish to call the attention of authors again to the advantage of well-prepared papers in respect to date of publication. If changes seem desirable, and if the paper must be returned to the author for approval of changes, it means delayed appearance of the manuscript.

They find the most difficulty with tables. Headings are incomplete, column headings often are obscure, and the form does not correspond with that used in PHYTOPATHOLOGY. A table should be as nearly self-explanatory as possible, for it is not always possible to place it immediately after the reference in the text. Examination of recent numbers of PHYTOPATHOLOGY would be helpful to many authors in determining what is required in tables, and would mean that their papers could be published earlier than they are.

Graphs also are frequently submitted which are unsuitable for satisfactory reproduction. When graphs on finely squared paper are reduced to page width the background often appears as indistinct grayish blocks. Plain paper or paper ruled in large squares gives more satisfactory results. All too often, also, letters and figures are so small that they can be distinguished only at the risk of permanent eye-strain. And they should never be pasted on.

Authors of scientific papers should be as accurate in expressing their ideas and the results of their experiments as they are in making their tests and in drawing conclusions. Clear writing—it need not be polished—is as much a part of “scientific accuracy” as the details of the experiments which the reader does not see.

A few avoidable errors have appeared in Volume 17: for these the editorial staff makes apology to the authors concerned.

REPORT OF THE ADVERTISING MANAGER

The advertising returns have been disappointing for the fiscal year of 1927. Instead of the increased financial returns we have experienced for the past two years, which were expected to be maintained, the record shows a decrease of business for the first time. A total of 113 advertisements appeared in the 12 issues for 1927 or a decrease of 27.5 per cent compared with last year. This total of advertisements was distributed as follows: 41 full pages, 39 half pages, 32 one-fourth pages, and 1 one-eighth page, making a total of 68½ pages of advertising or a decrease of 23½ pages. Three clients who have been placing a total of 30 pages failed to renew contracts for business reasons. If their business had been maintained it would have been possible to report another year of increased business.

It is desired further to impress the members that their cooperation is essential in order to increase our list of clients. When being solicited or when in contact with representatives of prospective commercial companies, make it a point to mention the merits of our publication as a practical medium in which to advertise. The personal appeal and contact is the most productive means of securing business. Our membership is sufficiently large and distributed so that with a little cooperation our business could easily be doubled.

REPORT OF THE ADVISORY BOARD

The personnel of the Board for 1927 is as follows: F. D. Fromme, Chairman, representative at large; F. C. Meier, Secretary, representing the United States Department of Agriculture; M. F. Barrus, representative at large; J. G. Dickson, representing the Midwest; B. B. Higgins, representing the South; J. E. Howitt, representing Canada; E. L. Nixon, representing the Northeast; and S. M. Zeller, representing the West. The following appointments were made by the Council for 1928: I. E. Melhus to succeed M. F. Barrus; J. A. Stevenson to succeed F. C. Meier; and F. L. Drayton to succeed J. E. Howitt.

F. D. Fromme was elected Chairman of the Board for 1928, and J. G. Dickson was elected Secretary.

Summer Meetings. The summer meeting for 1927 was held August 16–19 and included a tour of northern Ohio for the study of diseases of fruits, vegetables, and other crops. The local committee on arrangements consisted of H. C. Young, Chairman, W. G. Stover, Curtis May, Paul E. Tilford, and R. C. Thomas. The meeting was well attended and was unusually successful, due to the excellent program and arrangements provided by the local committee. The itinerary included the study of experimental

plots and commercial production of a considerable variety of crops such as potato, cucumber, tomato, corn, oats, onion, celery, raspberry, peach, and grape. Occurrence of disease on a number of additional crops was also noted. A more complete report of the meeting appears in *Phytopath.* 17: 836. 1927.

At the annual meeting of the Board it was decided to hold a summer meeting in 1928 on diseases of ornamental plants in the vicinity of New York City. A local committee on program and arrangements consisting of W. H. Martin, Chairman, L. O. Kunkel, C. R. Orton, W. H. Rankin, and L. M. Massey has been appointed, and plans are now being formulated.

National Research Council. The Division of Biology and Agriculture of the National Research Council has submitted a statement of the activities that are of particular interest to this Society, in order that the members may be informed of the types of service that may be rendered. A brief summary of the statement follows:

The Division through its various committees has aided the establishment of the Crop Protection Institute, the Tropical Plant Research Foundation, and the Barro Colorado Island Biological Station. It has given financial and other aid to *Botanical Abstracts*, *Biological Abstracts* and the Arthur Rust project.

It has had in operation since 1923 the National Research Fellowships in Biological Science. The fellowships of especial interest to this Society are the hard-seed fellowship which is in active operation under the direction of Dr. B. M. Duggar, and the sulfur fellowships which have largely been completed.

The Committee on Tropical Research has had under advisement the establishment of a graduate school of tropical agriculture and is now recommending Porto Rico as the most suitable location for the proposed school.

The Committee on Research Publications is determining the needs among scientific biological journals for the publication of original research.

"On behalf of the Division of Biology and Agriculture, its present officers wish to call the attention of the members of the American Phytopathological Society to the fact that the National Research Council welcomes the representation on it of the Society and that the Division is the unit through which the Society secures this representation and to whom it may bring any matters in which the Council can be of assistance. The extent to which this Society, along with the others included in this one unit, utilizes the Division organization as a means to accomplish some needs, is the extent to which the Division itself will justify its value to the societies."

Crop Protection Institute. The activities of this organization, which cooperates with the Advisory Board, are summarized from a report of W. C. O'Kane, Chairman of the Board of Governors, as follows:

At the close of 1927 the Institute had a staff of fifteen research workers, all but two of whom were full-time men. Fifteen research projects were in operation. These are listed briefly as follows:

Control of crown gall. At Ames, Iowa; Madison, Wisconsin; and Yonkers, New York.

Plant diseases carried by seeds. At Yonkers, New York.

Thallium and mercury compounds. At Yonkers, New York.

Insecticidal possibilities of the white oil emulsion, Volek. At Manhattan, Kansas.

Fungicidal action of a new colloidal sulfur. At Urbana, Illinois, and Madison, Wisconsin.

New colloidal copper compounds. At Yonkers, New York, with supplementary work in various states.

New compounds derived from furfural and furfuramid. At Ames, Iowa, and Fort Collins, Colorado.

Oil compounds as cattle sprays. At Lafayette, Indiana.

Insecticidal development of the household spray, "Kip." At Lafayette, Indiana.

Development of new oil emulsions. At Urbana, Illinois.

Development of the household spray, "Flit." At New Brunswick, New Jersey.

New horticultural sprays. At New Haven, Connecticut.

The physical character of atomized sprays. At New Brunswick, New Jersey.

Oxidized oils as insecticides. At Lake Alfred, Florida.

Organic insecticides. At New Brunswick, New Jersey.

Duties of the Advisory Board. The Advisory Board was formally established by the Society in 1918. A restatement of the duties as formulated at that time should be worth while. They are as follows: (1) To represent the Society before the National Research Council, (2) To prepare and distribute to the members an annual list of the active phytopathological projects in progress in the country, (3) To arrange for conferences of groups of workers on related subjects, (4) To confer with workers in related fields in order to promote joint efforts on our common problems, (5) To promote international relations in phytopathology, (6) To take up such other problems as the Board may find necessary, subject to the approval of the Council.

The recent activities of the Board, as will be noted, have dealt largely with the first, third, and fourth of these duties. No list of active projects has been prepared in recent years, and apparently there has been little need or demand for such a list. The Board has been, and is, successful in its chief function which is the promotion of co-operation. It will be glad to render any further service within its power that the Society may direct.

REPORTS OF OTHER COMMITTEES AND REPRESENTATIVES

Representative on American Type Culture Collection. In the absence of the Society's representative, C. L. Shear, the following report was submitted by Dr. Margaret B. Church. A supplementary list of available fungous cultures was distributed at the meeting.

"The first catalogue of the American Type Culture Collection, issued in 1927, lists about 230 fungous cultures in the group cared for by Drs. Charles Thom and Margaret B. Church of the United States Department of Agriculture. This group excludes human and animal pathogens and Actinomyces. Sixty-five per cent of the fungi included were contributed by Thom and Church, and the remaining 35 per cent by others. Since this catalogue went to press there have been added 130 new cultures now listed on a supplementary sheet. Of these new fungous cultures, 57 per cent were secured through the efforts of Drs. Thom and Church because of established personal relations, 24 per cent were secured through the efforts of their colleagues, 9 per cent were from their personal collection, while 7 per cent were from Dr. Shear, and 3 per cent from odd sources.

"It was the understanding of Drs. Thom and Church in December, 1924, that they should supervise constantly and critically the care and distribution of these fungous cultures after once formulating a standard of work. Accordingly, their efforts to secure cultures have been incidental to their official relations in the field of soil, food, and industrial mycology.

"An assistant is financed by the General Education Board and the Society of American Bacteriologists. The hours of work are arranged individually with each new assistant. The present incumbent, Mario Scandiffo, is a fourth-year medical student who

gives 21 hours a week service for the sum of eight hundred dollars. He transfers all cultures, keeps the records, and outlines the necessary letters.

"According to records in Washington, sales of fungi from January to December 10, 1927, numbered about 260, and seven cultures were given in exchange to parties who had contributed to the Collection. Sales of fungi were as follows: Aspergilli, 26 per cent; Penicillia, 18 per cent; Mucors, 19 per cent; Phytophthorae and Pythii, 10 per cent; and miscellaneous, 27 per cent. Of the miscellaneous cultures sold, 55 per cent were plant parasites, which means that, including the Phytophthorae and Pythii, 23 per cent of the fungi sold were plant parasites. At least 68 per cent of the sales were from fungous cultures contributed from the Thom and Church collection: namely, Aspergilli, Penicillia, Mucors, and miscellaneous. Therefore, 32 per cent of the sales were of fungi contributed by those not directly caring for the collection.

"According to records in Chicago for the collection as a whole, 3,897 cultures of micro-organisms were sold from a collection numbering 987. Three-hundred and seventy-seven institutions and 115 individuals were furnished cultures.

"The 1927 catalogue of all micro-organisms in the American Type Culture Collection and the cultures may be secured by addressing Dr. George H. Weaver, 637 South Wood Street, Chicago, Ill. To aid in meeting the expense of maintaining the collection, a charge of one dollar per culture, with addition for packing and postage, has been established."

Phytopathological Classics. The editors of *Phytopathological Classics* reported that they had two or three more papers in the series under way. These they hope to issue in 1928. The Business Manager reported that Classic Number 1 cost \$232.90 to manufacture. The income from sale of copies to date has been \$112.18.

Committee on Permanent Endowment. The committee on an endowment for PHYTOPATHOLOGY reported through its Chairman that it had been unable to come to an agreement as to the need for securing an endowment and so no active steps had been taken in that direction. On motion of J. G. Brown, of Arizona, it was moved that the committee be relieved of further responsibility and that a new committee be organized. An amendment was offered by W. H. Rankin, of New York, to have the committee consist of five members. The motion as amended was passed and the President later appointed E. C. Stakman, Chairman, J. G. Brown, H. H. Whetzel, A. J. Riker, and L. R. Hesler.

Auditing Committee. The auditing committee reported through its Chairman, W. H. Tisdale, as follows:

"We have examined the foregoing accounts of PHYTOPATHOLOGY and the American Phytopathological Society for the year 1927 and find them to be correct."

Elections Committee. The committee on elections reported that ballots had been counted and officers elected as reported in the first portion of this report.

Resolutions Committee. J. C. Walker, Chairman, submitted the following resolutions:

Resolved: That the members of the American Phytopathological Society hereby extend their hearty thanks to Professor Jesse M. Shaver and to the other members of the local arrangement committee for their faithful efforts in aiding the success of the Nashville meeting of the Society.

Resolved: That the members of the American Phytopathological Society hereby express their appreciation to the Nashville Chamber of Commerce for their generous help and cooperation during the preparation for and the holding of its 1927 annual meeting.

Resolved: That the members of the American Phytopathological Society hereby express their appreciation of the devoted attention and help received

during the Nashville session from Director W. W. Carpenter and Professors W. H. Yarbrough, C. MacMurry, and R. O. Beauchamp, of the Peabody Demonstration School, and their assistants.

Whereas, The American Phytopathological Society recognizes the great service that is being rendered by the U. S. Department of Agriculture in preventing or delaying the entrance of foreign plant parasites into the United States, thus affording invaluable protection to the plant growers of the Nation.

Whereas, The scientific and logical procedure is to supplement this service by investigating, in regions where they are now endemic, all natural and artificial methods of controlling those diseases and pests which have not reached or at least have not become widely established in the United States.

Therefore be it resolved:

1. That the American Phytopathological Society records its appreciation of the work of the U. S. Department of Agriculture in initiating field investigations abroad in cooperation with scientists in foreign countries for the purpose of studying potentially dangerous parasites already introduced, or likely to be introduced.

2. That the Society urge additional support for the extension of this important work, and,

3. That a committee of three be appointed by the incoming President of the American Phytopathological Society to cooperate with a similar committee of the Association of Economic Entomologists, with the Tropical Research Foundation, and with any other interested organizations in furthering this project in whatever manner seems most feasible.

Permanent Committee on Necrology. The committee reported the decease of two members during the year, Erwin F. Smith and P. J. O'Gara. The formal report will be found at the end of record.

Report of Special Committee Appointed to Edit Abstracts. The committee on editing abstracts for the Nashville meeting presented the following recommendation:

"That the closing date for receipt of abstracts be advanced to November 1, and that the committee shall be appointed and notified prior to that date.

"That the Secretary be authorized to return to the author for revision any abstracts which exceed the limit of 200 words, or which the committee considers not to conform to the standards of a meritorious contribution carefully prepared for publication.

"Returned abstracts may be resubmitted not later than November 20, but no paper shall be included in the program until it has been approved by the committee."

Committee on Plant Development. No report.

ACTION OF THE COUNCIL

In addition to making the appointments of the officers mentioned earlier in this report the Council submitted the following actions which were approved by the Society.

1. With regard to the matter of determining a policy with respect to sustaining life members who are delinquent in paying their dues, the Council instructed the Secretary to make such equitable adjustment as he may see fit.

2. The plans that have recently been announced in Science for the International Botanical Congress in London in 1930 were discussed to some extent and it was voted to ask Dr. Donald Reddick, who was special Secretary for our Society for the Ithaca Congress, to negotiate with the British Committee urging an adequate number of special sections for papers and business of strictly phytopathological subjects.

3. It was voted to provide for editorial and clerical assistance for the editors of PHYTOPATHOLOGY in an amount not exceeding \$300 for 1928. The University of Minnesota has contributed about \$300 per annum up until this year.

4. The recommendations of the special committee on the editing of abstracts were approved and recommended to the Society.

MISCELLANEOUS BUSINESS

At the conclusion of the paper by J. H. Craigie entitled "Heterothallism in the Rust Fungi," the following remarks and motion were made by A. G. Johnson:

"Mr. Chairman: I think we shall all agree that what Mr. Craigie and Dr. Buller have given us in the present paper is of far-reaching importance in many directions. Certainly this is an epoch-making contribution, in many ways ranking along with Debary's and Oersted's incontrovertible establishment of heteroeicism in the fungi.

"In view of this, Mr. Chairman, I would move that both Mr. Craigie and Dr. Buller be most highly commended for their important contribution, and I would move, further, that the necessary steps be taken to put forward Mr. Craigie's paper for very earnest consideration for the Association's \$1,000 prize."

The motion was unanimously carried and the Chairman (Dr. Overholts) after consultation with Professor Barss appointed a committee, composed of A. G. Johnson, E. C. Stakman, and R. J. Haskell, to recommend this paper for the prize. The recommendation to Robert J. Terry, Chairman of the Committee on Award follows:

"We are submitting herewith a paper by J. H. Craigie entitled "Heterothallism in Rust Fungi" which we believe deserves very earnest consideration for the American Association for the Advancement of Science's \$1,000 prize.

"This paper was presented Friday forenoon, December 30, before the joint session of the Mycological Section of the Botanical Society of America and the American Phytopathological Society. Following the presentation of the paper, the joint session voted unanimously that the paper be forwarded for very earnest consideration for the American Association for the Advancement of Science prize.

"We consider the paper of far-reaching, epoch-making importance because of the following reasons:

"1. It demonstrates that an organ which heretofore has been considered vestigial and functionless is of primary importance in the developmental cycle of the rusts, one of the most scientifically interesting and practically important groups of parasitic fungi.

"2. It demonstrates heterothallism (the presence of sexual strains) for the first time in the rust fungi.

"3. It furnishes a simpler and more reliable technique than has heretofore been available, for investigations of hybridization between species and parasitic strains of rust fungi, thus offering the possibility of studying experimentally the role of hybridization and consequent recombination and segregation in the origin of species and parasitic strains in this important group of fungi."

The Southern Division voted to make their next meeting a field meeting in some central location in the Southern States.

R. J. HASKELL, *Secretary.*

IN MEMORIAM

ERWIN FRINK SMITH, 1854-1927

Erwin Frink Smith was graduated from the University of Michigan in 1886, and received the degree of Sc. D. from the same institution in 1889.

From 1886 to the date of his sudden death, he was on the staff of the U. S. Department of Agriculture. For twenty-three years he was in charge of the Laboratory of Plant Pathology in the Bureau of Plant Industry.

For his monumental work on the bacterial diseases of plants, and particularly on the relation of some of these diseases to cancer in man and other animals, he received greater recognition than has been accorded to any American plant pathologist. He was sometime President of this Society, of Section G (Botany) of the American Association for the Advancement of Science, of the Society of Plant Morphologists and Physiologists, of the Society of American Bacteriologists, the Botanical Society of America, and of the American Association for Cancer Research. He was a member of the National Academy of Science. He received the honorary degrees of Sc.D. from the University of Wisconsin, and LL.D. from the University of Michigan.

He was a man of broad culture, deeply read in philosophy and in the poetry and literature of several languages. He was himself a poet of no mean achievements.

"Undoubtedly the future holds opportunities equal to those he saw and grasped. Undoubtedly there are others who will accept them, but not many who can do as he did."

PATRICK JOSEPH O'GARA, 1872-1927

Patrick Joseph O'Gara was graduated from the University of Nebraska in 1902, and received the honorary degree of Sc. D. from the same institution in 1917.

From 1902 to 1910 he was a member of the Office of Fruit Disease Investigations of the Bureau of Plant Industry, engaged principally in pear-blight control. From 1910 to 1914 he was plant pathologist and entomologist for the Rogue River Valley, Oregon, engaged principally in the control of pear-blight and frost injury. From 1914 to 1925 he was Director of Agriculture and Smelter By-Product Investigations for the American Smelting and Refining Co. at Salt Lake City.

He was the author of many papers dealing with plant pathology, entomology, agricultural engineering, and mathematics. His brilliant and restless mind ranged over a wide field of interest. He was a skillful mechanic, and expert electrical engineer, and the higher mathematics was his recreation. Essentially an engineer in outlook and methods, his work found expression in control methods now widely practised. Pending the publication of his extensive smoke-injury investigations it is impossible properly to evaluate his work.

REPORT OF THE NINTH ANNUAL MEETING OF THE CANADIAN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The meeting was held at the Dominion Rust Research Laboratory of the Agricultural College at Winnipeg, Manitoba, December 20-21, 1927.

The officers of the society for 1928 are as follows:

<i>President</i>	D. L. BAILEY
<i>Vice-president</i>	F. L. DRAYTON
<i>Secretary-Treasurer</i>	D. R. SANDS
<i>Members of Council</i>	J. DEARNESS and G. R. BISBY

D. R. SANDS, *Sec'y-Treas.*

ABSTRACTS

The reaction of wheat varieties to inoculations with Ophiobolus graminis Sacc.—R. C. RUSSELL.

Seventy-five varieties of wheat were inoculated in the greenhouse with a culture of *Ophiobolus graminis* Sacc., isolated from Saskatchewan field material. The inoculum consisted of a pure culture of the organism growing on moist, ground, oat hulls. Six grams of this was spread in each six-inch pot at seed level when the wheat was being sown. Every variety was tested three times, and a total of approximately 150 seedlings of each variety was exposed to infection. Each test lasted five weeks. At the end of this period the inoculated plants were stunted to about 40 per cent of the height of the checks, and about 60 per cent of them were dead. There were, however, slight but fairly consistent differences in varietal susceptibility noticeable. Good correlation coefficients were obtained between the percentages of dead plants of each variety in the different tests. The resistance shown by the strongest of these varieties was not considered great enough to warrant their use in fields infested with take-all.

Results of experiments on the control of barley stripe.—J. E. HOWITT and R. E. STONE.

Barley stripe, caused by *Helminthosporium gramineum* Rabh., causes very considerable losses in Ontario. The amount of stripe varies considerably in different years. In years when stripe is present, different varieties vary in the amount of stripe present. For the three years 1924-1926-1927 the average amount was as follows: Common six-rowed 16.33 per cent; Bearer (Ottawa No. 475) 6.67 per cent; O. A. C. 21, 3.33 per cent; Winnipeg No. 2, 0.33 per cent.

In our treatments the hooded variety Success was used, as it is susceptible to stripe. The following treatments were used. Semesan $\frac{1}{4}$ per cent solution soaked 2 hours at 22° C.; Semesan $\frac{1}{4}$ per cent solution soaked 1 hour at 45° C.; Semesan dust 3 ounces a bushel; Uspulun $\frac{1}{4}$ per cent solution, 2 hours at 22° C.; $\frac{1}{4}$ per cent solution, 1 hour at 45° C.; Uspulun dust 3 ounces a bushel; Dupont No. 12 dust 3 ounces a bushel; Bayer's dust 3 ounces a bushel; Copper carbonate 3 ounces a bushel; Vitriolene 3 ounces a

bushel; Formalin sprinkle 1 pint to 30 gallons of water. Dry formalin spray 1-1. The formalin sprinkle prevented germination. All the other treatments reduced the amount of stripe and also increased the yield.

Dupont dust No. 12 and the $\frac{1}{4}$ per cent solutions of Uspulun and of Semesan applied at 45° C. for an hour gave complete control. The other treatments were less effective.

It is necessary to continue these experiments in order to secure reliable results.

Reaction of Linum species of various chromosome numbers to rust and powdery mildew.—A. W. HENRY.

Linum species representing several groups based on chromosome numbers, for example *Linum grandiflorum* ($2\times = 16$), *L. perenne* ($2\times = 18$), *L. angustifolium* ($2\times = 30$ or 32), and *L. catharticum* ($2\times = 57?$) were used in these studies. Certain strains of *L. usitatissimum*, *L. angustifolium*, *L. crepitans*, *L. rigidum*, and *L. sulcatum* proved completely susceptible; while other strains at least of the first two species, as well as *L. perenne*, *L. austriacum*, *L. grandiflorum*, *L. flavum*, and *L. catharticum*, proved immune when inoculated with *Melampsora lini liniperda* from *L. usitatissimum*. *Melampsora lini cathartici* from *L. catharticum*, a rust supposedly confined to that host, also infected *L. rigidum* normally, but all other species of *Linum* tested were immune. An *Oidium* sp. which developed abundantly on all varieties of *L. usitatissimum* tested in the greenhouse at Cambridge University, England, also produced identical symptoms on *L. angustifolium* and *L. crepitans*. All other species tested proved immune except *L. perenne*, *L. austriacum*, and *L. rigidum*, which developed traces of infection but which are apparently highly resistant. The results of the inoculations with *M. lini liniperda* and *Oidium* sp. support previous evidence from crossing experiments and chromosome counts that *L. angustifolium* is the wild ancestor of *L. usitatissimum*. They also indicate that *L. crepitans* is closely related to these two species.

The haustorium of Cuscuta gronovii.—E. M. MOSS.

Haustral connections of *Cuscuta gronovii* Willd., with the following hosts were examined: *Monarda mollis* L., *Lathyrus ochroleucus* Hook., *Artemisia gnaphalodes* Nutt., and *Symphoricarpos racemosus* Michx. Uninterrupted union between the xylem strands of host and parasite is made by haustorial tracheids, as described by earlier investigators of *Cuscuta*; but connections between phloem strands of host and parasite by sieve tubes in the haustorium do not seem to occur. Haustorial hyphae, each with a single, hypertrophied nucleus, commonly penetrate the host phloem, where they effect considerable destruction of tissue and ramify in a coralloid fashion, some of the branches entering the host cells and others insinuating themselves amongst the cells. Other hyphae may penetrate series of cells in the cortex or in the pith of the host. The haustorium and stem of the parasite contain starch in abundance, while the host tissues have little, if any, of this carbohydrate. The enlarged penetrating hyphae commonly contain numerous minute starch grains.

Physiologic forms of wheat stem rust in Canada.—M. NEWTON, T. JOHNSON, and A. M. BROWN.

By greenhouse experiments, 24 physiologic forms of *Puccinia graminis tritici* have been shown to be present in Canada. Five of these forms are different from those reported by Stakman and his co-workers in the United States. Some evidence has been found suggesting a possible relationship between the varieties of wheat grown and the distribution of physiologic forms of rust.

In the course of determining the physiologic forms in 1927, thirty-three separate cultures of aeciospores from barberries, all but one of which had been artificially inoculated, were transferred to wheat plants in the greenhouse. In 14 of these wheat cultures, some greyish-brown pustules appeared. These were obtained in pure culture, and, as in the earlier case reported in *Phytopath.* 17: 711-725, 1927, they have remained constant in color, producing only greyish-brown uredinia. For most of these barberry infections, telia from *Hordeum jubatum* were used as a source of inoculum. (Dominion Rust Research Laboratory.)

Treatment of millet seed to prevent smut.—R. E. STONE.

Millet smut is a common disease of foxtail millets in Ontario, caused by the fungus *Ustilago crameri* Koern.

Seed from a badly smutted crop was treated in several ways. The untreated seed gave a crop 56 per cent smutted. Formalin sprinkle and formalin soak prevented germination of seed. Dry formaldehyde treatment markedly reduced the amount of smut but also checked germination to some extent. Bluestone soak and Bayer's dust were not effective. Semesan $\frac{1}{4}$ per cent solution and Uspulun $\frac{1}{4}$ per cent solution were quite effective, reducing the amount of smut to 1.9 per cent and 0.9 per cent, respectively. Dupont No. 12 dust reduced the amount of smut to 0.6 per cent, while copper carbonate reduced the amount to 1 per cent.

These experiments will be continued.

Sexual behavior of Puccinia graminis.—J. H. CRAIGIE.

An experimental investigation upon sex in the stem-rust fungus has demonstrated that it is heterothallic. The sporidia are of two kinds (+) and (-). A (+) sporidium gives rise to a (+) mycelium and a set of pycnia which produce (+) pycnospores. A (-) sporidium gives rise to a (-) mycelium and a set of pycnia which produce (-) pycnospores. When a (+) sporidium and a (-) sporidium are sown close together on a leaf, the (+) and (-) mycelia resulting therefrom intermingle and produce diploid aecia. When (+) pycnospores are brought into contact with (-) pycnia, or (-) pycnospores with (+) pycnia, diploid aecia are produced on the under side of the pustule receiving the pycnospores within a few days of the transference. There is therefore the possibility of two different strains of this rust crossing and producing a new physiologic strain of rust. The experimental results will be found in "Nature," Vol. 120, No. 3012 and No. 3030, 1927. (Dominion Rust Research Laboratory.)

Physiologic forms of Puccinia graminis avenae Erikss. & Henn., in Canada.—W. L. GORDON.

In 1924, Bailey reported the occurrence of five physiologic forms of *Puccinia graminis avenae*. Forms 1, 2, and 5 were found to be present in Canada.

More extensive collections of oat stem rust were made during 1925-1927. Forms 2 and 5 have predominated each year. Form 1 has been isolated infrequently.

A study of the heterogeneous or X reaction, given by form 5 on two differential hosts, was made by the single-spore method. The heterogeneous reaction occurred again, when inoculations were made with single-spore cultures.

Physiologic forms more virulent than 1, 2, and 5, first collected in 1925, have appeared each year. Form 3 has been collected once, but form 4 has appeared more frequently. A collection from Paskweggin, Sask., in 1925, yielded a form which differs in its infection capabilities from all other forms yet reported in being able to infect

heavily all differential hosts. It is considered new, and has been named physiologic form 6.

Seedling tests for rust resistance, of some 230 varieties or strains of oats from various sources in America, as well as more than 100 varieties from France, Germany, Sweden, and Russia, have been carried out in the greenhouse to physiologic forms 4 and 6. All were quite susceptible to form 6, and only one variety, from France, gave any indication of resistance to form 4. In a field test, these forms appeared to be equally virulent. (Dominion Rust Research Laboratory.)

A seedling blight disease of oats caused by Fusarium culmorum.—P. M. SIMMONDS.

The symptoms were described of the seedling blight of oats caused by *Fusarium culmorum*. Some observations on penetration and invasion of the oat seedling were reported. Penetration may take place through the mesocotyl or coleoptile. The fungus particularly invades the cortical tissues. Mycelium may collect between the coleoptile and plumule. At the crown, tiller buds may be invaded as well as root primordia. Some evidence has been obtained that entrance may take place through root hairs.

Sulphur dusting for the control of leaf and stem rust in Manitoba: Winnipeg small plot experiments.—F. J. GREANEY and D. L. BAILEY.

A series of one-fortieth acre plots was dusted with Kolo-dust at Winnipeg to determine the influence of the rate, frequency, and time of application of the dust on resulting rust control. A very heavy epidemic of leaf and stem rust developed during the season. The effectiveness of the method was conclusively demonstrated and the degree of control was in general proportional to the rate and frequency of the applications. Tri-weekly applications at 45 pounds an acre reduced the infection percentage of stem rust as compared with the check from 87 to 4 per cent and increased the yield from 12 to 48 bushels an acre. The only fortnightly application which was significantly effective was the 45-pounds-an-acre one and this did not compare favorably with weekly applications at lighter rates. From the practical standpoint it appears that bi-weekly applications at the rate of 30 pounds an acre or weekly ones at 45 pounds are most desirable for a bad rust year. The final choice will depend on the cost of application. Results indicate the desirability of beginning dusting as soon as traces of rust appear and continuing until the crop is practically mature. (Dominion Rust Research Laboratory.)

Sulphur dusting for the control of leaf and stem rust in Manitoba: Field trials with horse-drawn and aeroplane dusters.—D. L. BAILEY and F. J. GREANEY.

A horse-drawn duster applied 30 pounds of Kolo-dust an acre once a week for eight weeks on acre plots at Winnipeg, and the treatment reduced the infection percentage of stem rust as compared with the check from 90 to 40 per cent and increased the yield 20 bushels an acre. This result was as good as those obtained with bi-weekly applications at 15 pounds an acre. The net increase in value over the checks was \$15.28 an acre.

Aeroplane dusting was carried on in three localities. The results were splendid in one case, mediocre in a second, and negative in the third. The negative results indicated that in a bad rust year like 1927 dusting should be begun when only a trace of rust has developed, that 30 to 45 pounds an acre a week should be used and that dusting should be continued until the crop begins to mature. The positive results increased the value of the crop in one place by \$40.00 an acre. The aeroplane was thoroughly suited to this type of work especially on large continuous acreages. (Dominion Rust Research Laboratory.)

The dwarf leaf rust of barley in Western Canada (Puccinia anomala Rostr.).—A. M. BROWN and M. NEWTON.

This rust was first collected in Western Canada in 1922, but was not again found until this year when it was quite abundant in southwestern Manitoba and also at Indian Head, Saskatchewan.

Urediniospores were germinated in hanging drops of distilled water at different temperatures. The optimum for spore germination appears to be from 11° C. to 17° C.

A large percentage of urediniospores kept in the laboratory at room temperature, germinated at the end of a month, but at the end of three months no germination was observed. (Dominion Rust Research Laboratory.)

Cereal diseases in Alberta in 1927.—G. B. SANFORD.

The following are the chief results of the first extensive plant disease survey in Alberta. More than a trace of stem rust on wheat rarely occurs in Alberta. However, under the extremely favorable conditions of abundant moisture and a prolonged ripening period, an unusual amount of this rust developed over the entire area, from the northern limits of the survey (Athabasca) to the Montana boundary. Actual shrinkage occurred in the Camrose area, but very little injury elsewhere. Slight infection of stem rust of oats developed as far south as Lethbridge and north to Edmonton. Leaf rust of wheat was very prevalent and in many cases severe. Stripe rust (*Puccinia glumarum*) was found again at Olds on the leaves and glumes of wheat, and on nearby *Hordeum jubatum*; also in wheat fields and on *H. jubatum* in the southernmost part of the province. Glume blotch of wheat (*Septoria glumarum*) was very prevalent and severe. Slight infections of basal glume rot (*Bacterium atrofaciens*) and also of what appears to be black chaff of wheat (*B. translucens*) were collected at diverse points. Root rots of wheat were severe and are the most important plant diseases of Alberta, causing a loss estimated at 7,000,000 bushels. A distinct correlation of the severity of root rots with the black soil type was noted.

The Occurrence of yellow stripe rust in western Canada.—T. JOHNSON and M. NEWTON.

Stripe rust (*Puccinia glumarum*) was discovered at Edmonton, Alberta, in 1918, by Professor W. P. Fraser. Since then it has occurred annually on *Hordeum jubatum* in certain localities in Alberta. On barley it has been found once only. On wheat it was first observed in 1926 at Olds, Alberta, and again in 1927. Other hosts found naturally infected are *Agropyron smithii* and *Agropyron tenerum*. The area in which stripe rust has been found now includes scattered localities from Edmonton to the international boundary as well as southwestern Saskatchewan.

Attempts were made to determine whether the rusts on *H. jubatum* and on wheat were the same form or two distinct, specialized forms. Difficulty was experienced in establishing greenhouse cultures of the rust, probably on account of poor spore germination. Finally two cultures were established, one from wheat, the other from *H. jubatum*. Both of these proved to belong to the *P. glumarum tritici* form. Although this does not prove that only that form exists in Canada, it shows that the rust on *H. jubatum* may under favorable conditions affect wheat.

Owing to difficulties in obtaining artificial infection, an attempt was made to determine optimum conditions for germination of fresh urediniospores produced in the greenhouse. Various methods were used at 10° C., 15° C., 17° C., 19° C., and 23° C., but under all conditions tried germination remained low and irregular. Germination was best at 10° C. and 15° C., the average being only about 8 per cent in each case. (Dominion Rust Research Laboratory.)

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BLIGHT-RESISTANT POTATOES

DONALD REDDICK

The primary purpose of this article is to make a second report of the progress that has been made in the development of a potato (*Solanum tuberosum* L.) that is resistant to the disease known as late blight or, more commonly, blight [caused by *Phytophthora infestans* (Mont.) deBy.]. A short discussion of biologic specialization and of the origin of the parasite is added.

Since the appearance of the first brief report on this subject by the writer (41), extensive reports on a similar endeavor instituted by Broili (11) in Berlin have appeared. K. O. Müller (35), in particular, has summarized the early work in breeding for disease resistance and especially for blight resistance, in potatoes; and Appel (4) has published a more popular account of the same sort. In view of the fact that the work is scarcely known in the United States, these authors and others may be pardoned for not including in their reviews a reference to one very important effort, and perhaps the first one, to overcome blight by breeding methods. Almost as soon as blight became a problem in the United States, Chauncey E. Goodrich (19), of Utica, New York, began work which extended over a period of nearly 20 years on the "regeneration" of the potato. Although Goodrich never admitted the causal relation of *Phytophthora infestans* to the blight, his stand on this subject coincided with that of nearly every other person in the world who had an opinion on the etiology of blight. If this one error is overlooked it will be found that Goodrich's observations on the occurrence and spread of blight, his description of the disease in all its phases, and his observations on the attendant conditions of an outbreak are extremely accurate in every detail. Goodrich made a very positive contribution to potato culture by the introduction of new "blood" into an old line; a contribution which is fully acknowledged by Salaman (45, p. 7) in his recent book, "Potato Varieties." Furthermore, Goodrich's contribution was particularly important in that he showed definitely that races of potatoes exist

which are highly resistant to the disease, that these "wild" potatoes can be ameliorated by hybridization with the commonly cultivated sorts, and that resistance is transferred to a part of the progeny. While it is not perfectly certain, there are strong indications that the variety Evergreen, which is now cultivated to a certain extent and which is so resistant to blight that a good crop can be secured even in a year of severe blight, is one of Goodrich's hybrids.

Evergreen has been sent to the writer repeatedly under the name of "blightproof." It is also the variety which is grown in New Jersey under the name "Red Skin," and it is also the variety which Jehle (25) has recently recommended to Maryland potato growers as a substitute for McCormick. Jehle's very recent record (26) of as high as 25 per cent of *Phytophthora* tuber-rot in "Red Skin" indicates that there may be several closely related varieties known by this name. So much rot of tubers has not been found at Ithaca where tests have been made on several lots, including one sample from Doctor Jehle.

Evergreen is well named in that the variety is highly tolerant of dry weather and forms a very large green vine when such varieties as Green Mountain or even Rural are severely injured. It is by no means immune to *Phytophthora*. In a four-group classification of plants in which 4 represents as complete absence of resistance as is exhibited by Green Mountain, Evergreen is usually listed at 3, although some strains have been tested in 1927 which may be listed at 2. The variety often develops seedballs when grown in the field with other varieties, but all attempts (several hundred) to self the variety in greenhouse or field have failed. It seems to be completely male sterile, and no pollen grains that appeared to be normal ever have been seen. Viable pollen of several varieties has been tested on Evergreen, and seedballs have been produced. When pollen of a homozygous variety (Ekishirazu) is used, the first generation shows such a heterogeneity that Evergreen is certainly heterozygous. The genetics of *Phytophthora* resistance is difficult because homozygosity is impossible to obtain with a large number of varieties which are male sterile. Even in certain wild species of *Solanum* an incompatibility exists which makes selfing very difficult or even impossible. Genetical work in collaboration with C. H. Myers, of Cornell University, is in progress but will not be reported at this time further than to say that resistance to *Phytophthora* is a heritable character, at least in certain instances, as will be apparent from subsequent remarks.

The prime requisite in breeding for resistance to *Phytophthora* is, of course, the existence of a resistant stock. Fortunately this is at hand in Ekishirazu (blight unknown or blight proof), a variety which was described by Ito (24).

It would be possible to use Ekishirazu directly in commercial culture, at least in the Northern States and Canada, if the variety were suitable for the purpose. Unfortunately it is not suitable. The variety has not made a good growth at Ithaca, New York, in any of the five years that it has been under test. The plants in the field are very short and prostrate, although they have been grown in the greenhouse simultaneously and commonly attain a height of 2 meters or more, and the tubers rarely have reached marketable size. In addition, the rough, scaly skin is objectionable, and the flavor is not all that could be desired in a good commercial sort. The resistance of the variety to blight is its most commendable character. This resistance is not absolute. It is always possible in the greenhouse to secure infection on the very young leaves at the tip of the stem. The tip of the stem itself becomes infected too but the lesion produced rarely extends as far as 2 centimeters down the stem. Lesions also may appear on older leaflets, but these usually are not more than one millimeter in diameter although they may attain a diameter of 3 mm. Leaf lesions have not been observed in field-grown plants, and the stem-tip lesions are very uncommon. Furthermore, the fungus rarely fruits on these lesions even when affected parts are placed in a humid chamber. Aerial mycelium is developed under such conditions, and in some instances sporangia have been found. When a number of lesions develop on a single leaflet or when one or two lesions develop on several leaflets, the whole leaf is very likely to turn yellow and fall from the plant. The incubation period is practically identical with that on such a susceptible variety as Green Mountain; at most, it is only a few hours longer on Ekishirazu. The variety possesses a very high degree of resistance, and if it were grown commercially at Ithaca there would be no loss from blight even though susceptible varieties proved a total loss when grown alongside. Plants approximating this condition are given a rating of 1 in tests for resistance. A plant that can be rated 0 is still sought.

Ekishirazu, fortunately, flowers freely, is self fertile, and produces seeds very readily. When flowers of this variety are selfed they do not always set fruit, but when the pollen of Ekishirazu is used upon any one of a number of commercial sorts a set of fruit is almost certain to result. Seedlings of selfed Ekishirazu, when grown and inoculated with *P. infestans* in a flat in the greenhouse, were somewhat susceptible. All of the plants at the time of inoculation had four to six leaves, all of which were of juvenile (entire) form. A few plants were killed. Those remaining, whether affected or not, were moved to the field. The blight did not spread on any of the plants, nor did the plants become affected when inoculations (both artificial and natural) were made on them later in the summer.

Evergreen and Ekishirazu have been described in some detail not only because they are not widely known but also because hybrids of them have

yielded the largest percentages of resistant plants of any of the combinations thus far attempted. A few other varieties may be mentioned briefly for certain characters that have made them useful in blight-resistance work. Irish Cobbler, as a female parent, when crossed with Ekishirazu has yielded a goodly percentage of promising plants. This variety, it may be noted, traces back through Early Rose and Garnet Chili to Goodrich's Rough Purple Chile. Green Mountain, the various Rurals, American Giant, Up-to-Date and the like, when used as female parents, have proved very disappointing. Some varieties which were secured in the Vosges of Alsace in 1924 and which were selected because of their very marked resistance to blight (single green plants standing in potato fields that had been completely destroyed by *Phytophthora infestans*) give evidence that they may prove valuable as foundation stock for breeding purposes. The names of these varieties can not be ascertained. One of them is known locally as Steinthaler, but this is only the name of the valley in which it grows and is not a recognized varietal name in European literature. Another is called Halbfrüh, but here again the name is one to indicate date of maturity and is not a known varietal name. These plants grew very satisfactorily at Ithaca in the summer of 1925 and of 1926, but in 1927 the dry and warm (at times) weather affected them as seriously as, or more so than, Green Mountain, a variety which is markedly affected by such conditions. Their usefulness therefore remains problematical.

Early in the work, first generation hybrids were tested in the seedling condition for *Phytophthora* resistance. Practically all the seedlings became affected, and it was tentatively assumed that if resistance were a heritable character at all it must be transmitted as a recessive. When the seedlings of Ekishirazu showed infection, this opinion had to be revised; and it is now known that it is worth while to make field tests on hybrids of the first generation. In some cases this is highly important since the hybrids may prove to be male sterile and in such cases a second seminal generation can not be obtained. It is worthy of note in passing, however, that plants on which no seedballs have set after several hundred trials may for no apparent reason produce one or more flowers which self readily and which produce good seeds. Most of the tests on which this report is based have been made with second generation hybrids, although a few involve first generation hybrids and a few others those of the third generation.

Thanks to the work of Melhus (33), and now to the extensive work by Vowinckle (51), the method of securing infection with *P. infestans* is well understood. No difficulty is involved in the greenhouse where external conditions can be controlled. But since greenhouse tests with seedlings are not reliable, and since several thousand plants have been tested each year, it has

been easier to run the tests in the field. Under field conditions it is not always so easy to secure infection. It happens that, during the three years 1925 to 1927, inclusive, seven or eight separate inoculations have been made each year before the blight became satisfactorily abundant in the field so that a uniform natural infection could be expected to occur. Thus far the classification of plants into categories of resistance has been based upon a natural spread of the disease from alternate rows of susceptible plants which became infected following artificial inoculation. It is perhaps possible to make eliminations after an artificial inoculation but there is always some risk that really desirable plants will be discarded. It has usually happened that the field inoculations have been made just at dusk and there has been no time to regulate the number of swarm-spores that were applied to each plant nor to see more than that each plant received some of the inoculum. Under such conditions certain leaves of a plant are likely to receive an excessive number of viable spores and to be destroyed by the fusing of very many small lesions. It also happens frequently that the progress of the lesion on a leaflet and the abundant production of conidia indicate that the plant ultimately will be classed as completely susceptible. Subsequent events, however, show that only the affected leaflet is involved and that the leaflet either becomes dry or perhaps the whole leaf yellows and falls. The fungus does not spread down the midrib of the leaflet and involve the other leaflets nor the stem. In other cases the number of penetrations on a resistant plant is very small. Intermingled leaves of a susceptible plant may show dozens of separate infections, whereas only a few infections occur on the resistant plant.

In 1925 the work was cut short by frost. Every plant that showed indication of resistance was saved for further test. In 1926 conditions were ideal for the test: a better year for such work never can be hoped for. During the month of August there were 26 days recorded as cloudy or partly cloudy, with precipitation on 20 days and with minimum temperatures of 13° C. or lower on eight nights as follows: 9, 10, 21, 22, 26, 28, 30, 31. In September there were 23 cloudy or partly cloudy days, there was precipitation on 15 days, and there were 21 nights with minimum temperatures of 13° C. or lower. The plants were not killed by frost until the night of October 16. Of the 16 days, 14 were cloudy or partly cloudy, there was precipitation on 12 days and the minimum temperature was at or below 13° C. on 13 nights. In 1927 the conditions were not so good and were complicated by the fact that many lots of plants were injured by extreme drouth. So far as commercial production is concerned, however, the season made possible the elimination of drouth-susceptible plants. The last artificial inoculation of 1927, nevertheless, was made under ideal conditions—a great quantity of

swarmspores in condition to germinate at once, a very fine misty rain setting in before the inoculation was completed, and the night one of fog and mist. Every plant used as a check became infected, and this meant every other row in a field of more than two acres. The natural infections occurred subsequently, one of which was extremely heavy and another one not so heavy, the latter due in part to the fact that some of the susceptible plants were already dead, thus reducing the quantity of inoculum. The yield in 1927 was very unsatisfactory but this condition existed in most fields in the vicinity and is attributable to dry weather. Each year most of the plants that graded 1 or 2 in a scale of 4 were saved for further test, but plants exhibiting obviously undesirable characters such as dwarfing, frenching, evidence of extreme drouth injury and the like, either in vine or tuber, were discarded at harvest time. The suggestions of Stuart (47) about the kinds of seedling tubers that are worth propagating beyond the first clonal generation were kept in mind but were not applied at all rigidly. It was desired to carry through several generations a considerable number of lots that exhibited high resistance in order to see whether any or all might lose their resistance in the way that de Candolle (14, 15) reports for *Solanum verrucosum* Schlecht.

There exist nearly 300 separate lots of plants for most of which there is a definite record of blight resistance for the two consecutive years 1926 and 1927 with the general record that these plants exhibited decided resistance (grade 1 or 2) in 1925. Upon tabulation it appears that 43 lots of plants have been given a grade of 1 for the two years and 116 have been given the grade 2. Eighty-six lots that were marked grade 2 in 1926, a highly favorable season for blight, were marked grade 1 in 1927, a less favorable year for blight. On the other hand 12 plants of grade 1 in 1926 dropped to grade 2 in 1927, and 19 plants of grade 2 dropped to grades 3 or 4. These records will be supplemented by behaviors in 1928 and will be further supplemented by 272 plants which have been graded 1, and 289 that have been graded 2 in 1927, as well as by over 2,000 new lots which are to go into the field for the first time in 1928.

From the foregoing record it appears fairly well established that resistance to *Phytophthora infestans* in these various lots is rather definitely fixed. In fact it is entirely conceivable that their resistance does not vary at all but that the writer's ability to classify degrees of resistance accounts for all of the variation. Such a classification is much less definite than, for example, one involving the amount of rust on a blade of wheat. After passing judgment upon a few hundred plants one acquires a certain discrimination which is useful for the remaining plants. At the end one must return to the first hundred or more plants in order to give them a fair and comparable rating.

Certain anomalies have existed throughout the work. Occasional instances have been noted in which a certain lot of plants has been classed 1 throughout a whole season, only to drop to 3 or 4 the next year. The reverse of this has not been observed because a plant rated 3 or 4 has been discarded. While it would be easy to say that such plants have lost resistance, it would be difficult to prove it, and for the present the statement that the plants simply escaped infection fortuitously is just as likely an explanation.

Another almost anomalous condition which is evident only in the field notes is one in which a plant is recorded as showing resistance 3 and after a few days is marked up to 2 or even to 1. The rapidity with which a few leaflets are being destroyed leads to the assumption that the whole plant will be killed, whereas these few leaflets very soon die and the number of new infections from period to period is so small that careful examination is required to find that the disease exists there at all.

Still another condition has been observed which is very interesting. Certain very large plants reached a condition in late summer in which the lower leaves were so much shaded that they turned yellow and eventually dropped from the plant. Following a particularly heavy natural infection in 1926 it was found that the green foliage of some such plants was entirely free from lesions or, in other cases, at the most, showed only very small spots about 1 mm. in diameter. The senescent leaves, however, were attacked and the fungus could be found fruiting upon them. No definite lesion was evident, and the sporangiophores were so scattered that their presence would not have been suspected except for the fact that each one bore a droplet of dew. This condition is of interest from both the theoretical and from the practical standpoint and is one that must receive further study. Vowinckle (51, p. 616) presents data for a susceptible variety which anticipate this result in that he found susceptibility increasing with the age of the host and a higher susceptibility of the basal leaves of a plant than of those higher up. Miss deBruyn (12) also found the same condition existing when susceptible varieties were inoculated in the field, and thus differs with Pethybridge (39), who concluded that there was no material difference in susceptibility of plants in different stages of development.

Another condition that has been observed, clearly not an anomaly, is that foliage may show a high degree of resistance while tubers of the same plant exhibit little or no resistance. This is the antithesis of the condition described by Miss Löhnis (32) for the variety Bravo. No systematic test of tubers for resistance has been made as yet, and this mention of tuber susceptibility is based only on those occurrences of natural infection that have been encountered. The presence of affected Green Mountain foliage,

often intermingled with the healthy foliage of resistant plants, furnished abundant inoculum, and the destruction of Green Mountain tubers from rot has ranged from 50 to 100 per cent. A hill of Green Mountain that was free from tuber rot scarcely ever has been found, and when one such was found it was usually evident that the plant had been killed before blight became prevalent in the field. Since this condition was general throughout the field and since the number of lots of resistant plants showing tuber infection has been small, even under conditions extremely favorable for *Phytophthora* rot, it is assumed, tentatively, that resistance to infection in tubers is often coupled with resistance in foliage. From the practical standpoint it is obvious that tuber susceptibility is not a matter of great importance provided the foliage shows a resistance of grade 1, *i.e.*, so resistant that the pathogene is not able to perpetuate itself.

The susceptibility of fruits is of interest too. Not many records are available, but those that are show that seedballs on susceptible varieties are very susceptible. Those on Evergreen (resistance grade 3) apparently are as susceptible as on Green Mountain (grade 4), and, interestingly enough, those half-dozen or more seedballs which were produced in the field on a plant of grade 2 seemed to have no resistance at all. Unfortunately no records are available on the condition exhibited by seedballs on plants of grade 1. It is entirely possible that they have no resistance either, just as Lesley (30) has found in *Solanum edinense*, in which case a perfect transition would be effected to the tomato and finally to the egg-plant. This will be brought up again in another connection.

Very few, if any, of the various lots of the hybrids are exactly alike. All sorts of variations exist and it should prove possible to select for a considerable range of requirements. There are even indications that early sorts may be found, although this was not anticipated and as yet no adequate test has been made. Likewise no cooking tests have been possible with the resistant plants. A great many of the susceptible sorts have been given a cursory test and in the vast majority of cases the hybrids have proved of better quality and flavor than their resistant parent. Records on conditions after storage are available but are not particularly significant as yet because of the small amount of tubers available for test.

It is the intention to select several of the more promising sorts for rapid increase so that samples may be distributed widely for testing under a great variety of conditions. The plan also includes a test of certain of the most resistant sorts in a number of foreign countries, not so much with the idea that the plants may prove adaptable to local use in commerce as to find out whether *Phytophthora infestans* is biologically specialized. The advantage of investigating this problem under identical conditions of growth of the

host is appreciated, but on the other hand the difficulties of securing the necessary material for tests at Ithaca are too well known to require enumeration.

Likewise there is a positive advantage in conducting such tests under dissimilar conditions. Until the discovery of the existence of biologic specialization, it was conventional to assume that differences in behavior of a given host to a specific disease under widely differing conditions was occasioned by the effect of the environment on the host. Biologic specialization has been found to furnish a more satisfactory explanation of some of these cases but it does not follow by any means that it is always the explanation. One has only to inoculate potato plants with *P. infestans* under glass and in the field to know that environmental factors do make a difference in the amount of disease that results. Persons who are interested in securing plants or in extending this investigation in any possible manner are invited to make their wishes known.

BIOLOGICAL SPECIALIZATION

In the absence of definite information on the subject, it has been assumed for the purpose of this work that biological specialization in *Phytophthora infestans* does not exist. The assumption, however, has not been made without some foundation in fact. Indeed there has been very little found out about this organism which would indicate the existence of high specialization. The difficulty of bringing the fungus into pure culture on an artificial medium has been made the basis for an assumption of approximate obligate parasitism and a high specialization which the recent work of Miss deBruyn (13) has done much to dispel.

Likewise the infection of senescent foliage recorded earlier in this paper points to a rather low order of parasitism, as does the fact that the fungus kills its host outright. In the same way the wide host range of *P. infestans* argues against a high degree of specialization. The fungus is reported to occur on several different Solanaceae, some of them rather far removed from the Tuberarium group, and two of them (*Schizanthus* and *Anthocercis*) so far removed that in older taxonomic works they are to be found listed with the Scrophulariaceae. Some of the species affected are: *S. utile* Klotzsch (Münter, 37); *S. verrucosum* Schlecht. (de Candolle, 15); *Solanum dulcamara* L. and *Schizanthus grahami* (deBary, 5); *Anthocercis viscosa* (Berkeley according to deBary, 5); leaves and fruit of *S. muricatum*, *S. caripense* Kunth, and *Petunia* hybrids (Lagerheim, 29); *Lycopersicum esculentum* (Payen, 38 and Berkeley, 7); *S. maglia* Molina (Darwin ?, 17 and Sutton, 48); *S. demissum* Lindl. and *S. cardiophyllum* Lindl. (Lindley, 31); *S. edinense* Berthault (Salaman, 44); *S. aviculare* (Berg, 6); *Lycium*

halimifolium, *L. turcomanicum*, *Solanum nigrum*, *Physalis alkekengi*, *Hyoscyamus niger* (Vowinckle, 51), the last four only on detached leaves. In this laboratory infection has been secured on *S. commersonii* Dunal (3 samples from coastal plain of Southeastern South America); *S. maglia* from the island of Chiloé, Chili; *S. fendleri* Gray and *S. jamesii* Torrey from Southwestern United States. *S. dulcamara* has become infected repeatedly by inoculating with the fungus taken from cultivated potato. Just as described by deBary (5), the lesion produced is very small, and the leaf soon yellows and falls, but *P. infestans* is present abundantly and fruits freely on affected leaves which are put in a humid place. *Solanum nigrum*, *S. carolinense*, *Physalis alkekengi*, *Hyoscyamus niger* and some other solanaceous hosts of uncertain identity did not become infected either in greenhouse or field. No trials were made with detached leaves. Leaves of *S. melongenum* also have become infected when the host has been weakened by exposure for three days in a humid chamber with weak light. Haskell (21) has reported the occurrence of lesions on the calyces and fruits of this host which he attributes on strong circumstantial evidence to the *Phytophthora* from potato. Salaman (44) reports that *S. edinense* was highly resistant in his cultures for 20 years but that suddenly, with the resumption of the sexual process of reproduction, the species became susceptible. This seems to be a revival and extension of the "principle of compensation" which was defended by de Candolle (15) years ago, and which has been invoked subsequently on many occasions, particularly by Jones (28), who speaks frequently of the "critical period" in the life of the plant. Pethybridge (39) has done much to dispel this idea of a critical period and the weakening of resistance at the time of flowering and of tuber formation. The circumstance reported by Salaman certainly suggests the introduction of a new biologic form of the fungus into his cultures, but there seems to be no way of testing this point now unless *S. edinense* of the same clonal line can be found which has not yet resumed sexual activity.

It may be contended that a fungus does not have to be a highly specialized parasite in order to exhibit the phenomenon of biologic specialization. That this may be true is indicated by the recent and extensive work of Berg (6). Berg's experiments are so numerous and so comprehensive that his conclusions seem fully justified. Berg used in his experiments the tomato variety Stone, a fact communicated by him in correspondence. For the purposes of his work the variety name is not important, but in comparing his work with that of Bondartseva-Monteverde (8, 9) one is led to the conclusion that the latter worked only with that fungus which Berg would call the potato strain. Nothing in Bondartseva's paper can be taken to indicate that she was dealing with specialized forms. While it seems im-

possible to place any other interpretation on Berg's experimental data than he does himself, nevertheless the following sentence in his "Field Observations" seems to constitute a paradox. His statement is (p. 27): "In instances where the early development of the blight was kept under close observation, the first signs of tomato blight were found on the plants adjacent to potato plants that were heavily infected with *P. infestans*." In his summary he makes the following statement which is even more significant: "In all cases observed under natural conditions, potato late blight appeared earlier in the season than the tomato late blight and wherever tomato late blight was found potato late blight also occurred in close proximity."

An epiphytotic of *Phytophthora* blight and fruit rot of tomato has occurred in the southern coastal counties of California in the autumns of 1927 and—to a more limited extent—1926 as reported in personal correspondence with G. B. Ramsey and I. C. Jagger. The latter upon request has made some cursory inquiries and upon rather satisfactory circumstantial evidence suggests that the same organism is probably causing the blight of both potatoes and tomatoes in this region. Some comparisons of the tomato fungus from California with the potato fungus from Ithaca have been made. The only difference so far observed is an apparently greater virulence both on tomato and potato of the California fungus.

So far as the North American continent is concerned, it seems that any specialized race of *P. infestans* on potato would be very widely distributed, particularly in view of the fact that the potato has been in cultivation on the continent for less than 300 years, that in many places its culture is not yet 50 years old, and especially in view of the extensive movement of planting stock from the extreme North to the extreme South and of table stock from the South northward, not to mention a certain inter-regional movement between East and West, especially in times of crop shortage in some region.

In the work here reported no attempt has been made to use a single strain of *P. infestans* for inoculation purposes. In fact, cultures or infected tissue have been secured from various places in New York, from West Virginia, and from Florida and used in making inoculations. It would have been easy to use pedigree cultures for tests made in the greenhouse. In the field, however, there is no way to prevent promiscuous infection, and there is every reason to believe that such infections have occurred at least in 1926 and in 1927. Nothing in the behavior of the potato cultures indicates that a new strain of the parasite has been introduced. Certainly the very small number of cultures that dropped in their recorded resistance can not be interpreted as an indication that this has happened.

If the question of specialization is considered from the intercontinental standpoint, it may be noted that Ekishirazu is equally as resistant in New York as in Japan. On the other hand a sample of Ekishirazu was sent to A. H. Cockayne in New Zealand and was pronounced (letter) susceptible by him. The only uncertainty about this evidence is that the tubers originally sent to Cockayne by the writer were relatively smooth-skinned, whereas Ekishirazu as grown at Ithaca usually has a rough skin. More typical samples were subsequently sent but no report on their behavior has been received yet.

Substantiating evidence for the occurrence of specialization may be seen in the fact that the seeds of Ekishirazu were obtained originally from some other country than Japan, possibly from France, but in any event a variety possessing the resistance of Ekishirazu was not known anywhere at the time those seeds were bought. This easily leads to the assumption that in some other places Ekishirazu would not prove resistant. So far as is known no test has been made.

In view of the well-known fact that blight very commonly appears first in Western Europe on the Atlantic coast and that it spreads rapidly eastward, it would seem that no biologic specialization is to be expected in Western Europe. The place to look for specialized forms would be far inland, as for example in Russia. Such evidence as exists for Russia, however, is (Bondartseva-Monteverde, 8, 9) that no specialization exists. If it should happen even occasionally that the spread of blight in Europe should be reversed this reasoning would be faulty. No evidence on this question has been found in the literature of the subject, voluminous as it is.

ORIGIN OF PHYTOPHTHORA INFESTANS

The origin of these resistant and semi-resistant plants is a most interesting subject for speculation. It was Goodrich's idea that he should go to the land where the potato is endemic to find plants with which to regenerate the common cultivated potato. This may have been an original idea with Goodrich but more likely, perhaps, was acceptance of the generally prevalent idea credited by Salaman (46) to Parmentier in 1786, that the potato had degenerated because of repeated asexual propagation. At any rate the thought antedated by several years the most gratifying demonstration (from the standpoint of plant pathology) of the soundness of the idea, namely, the success in combatting *Phylloxera* of the grape by the use of wild stocks from North America. For Goodrich, and those who denied the causal relation of *Phytophthora infestans* to "the disease," the reasoning is good, but for the modern pathologist no analogy exists. The French went to the land where the parasitic *Phylloxera* was endemic, and to make the

analogy perfect it must be assumed that *Phytophthora infestans* is also endemic to the higher Andes of South America. Upon looking for evidence that blight was known to the Indians or to the Conquerors it appears that practically none exists. Cieza (16) who mentions potato culture of the Indians does not mention any disease of the crop. Münter (36), as well as Vowinckle ('51), cite a passage from Acosta (1) which is interpreted to mean that Acosta had observed potato rot in South America, presumably at Juli on Lake Titicaca where he spent most of his life. The word used by Acosta is "anublan." If a contemporaneous Spanish dictionary is consulted it will be seen that this verb might be used technically to indicate the production of injury by the burning of tissue by the sun's rays when passing through droplets of water. Acosta's whole "history" is free from technical terms, and it seems unlikely that he should have used this one word in a technical sense. Furthermore the context leads one to believe that he meant nothing more than that the potatoes sometimes "spoiled" in the ground because they became frozen. This is the meaning given by contemporaneous translators (Regnault, 1600 and 1616 and Grimston, 1604) of the *Historia*. The Acostan "evidence" proclaimed by Boussingault (10) refers to another Acosta entirely—an army colonel who was resident in Colombia at the time. It is extremely difficult to see how the colonel's testimony adds anything whatever to our knowledge of the disease. The only words in the letter, as published, that bear on the question are the following: "La maladie . . . est une espèce de champignon ou excrescence qui se développe sur différents points, et qui corrode plus ou moins profondément ces tubercules." Garcilasso de la Vega (18) makes it perfectly clear that the potato is "inclined to rot soon on account of its moistness." This statement is made in a chapter which treats of the storage depots for provisions and is part of a description of the freezing and drying process to which potatoes were subjected in order to make chuño, a food material which can be stored indefinitely. This author had some knowledge of crop production in the Inca country from personal contact in the management of the vast estates of his father. He mentions potatoes in five different places in his *Commentaries* but never mentions disease. It must be inferred from this that the disease did not occur anywhere in the vicinity of Cuzco during several years at the middle of the sixteenth century. The Lagerheim (29) evidence (1890) is unmistakable but as this is of very recent date it is entirely possible that *Phytophthora infestans* was brought to Quito, or at least to South America, on European potatoes or other solanaceous hosts.

It seems a matter of some significance, too, that no record has been made of the occurrence of *Phytophthora* on specimens of South American Solanaceae collected prior to 1845. The natural inference is, considering that

every botanist in Europe was actively concerned with the disease and was serving on some national commission for investigating it, that all the sheets of tuberous Solani were critically examined for evidence of the disease and that if any lesions even remotely resembling blight had been found they would have been reported.

In viewing the matter from another angle, it will be recalled that deBary long ago pointed out that the potato might not be the natural host of *P. infestans*. He based this idea on the fact that the fungus does not form oospores on the potato, whereas practically all other members of the Peronosporae do produce their oospores on the plants that they attack. DeBary searched for oospores on *Schizanthus grahamei* without success.

The failure of *P. infestans* to reach Europe until nearly 300 years after the potato was introduced is likewise an important support to the idea that the fungus is not endemic in South America. The well-known explanation of Jensen (27, pp. 154-156) to account for this situation has been siezed upon eagerly and embraced by everyone who has need for some explanation of this circumstance. At the outset it may be assumed that potatoes were rarely brought to Europe except by accident. There was no demand for them and there is no likelihood that they would be carried as ships' stores except in cases where provisions had to be taken on in South America or from some captured vessel. When potatoes finally became an article of diet, however, active interest was aroused in the growth of the crop, and one of the most popular remedies proposed for the suppression of the widespread disease known as curl was to send to South America for new stock.

For many years, and in particular throughout the seventeenth century, every Spanish port of South America was closed to transoceanic traffic except Nombre de Dios on the north shore of the Isthmus. Prescott (40, Bk. 2, chapt. 3) cites manuscript sources to show that Pizarro himself, on his second attempt to reach what is now known as Peru, subsisted in part upon potatoes. A few years later Cieza (16) found potatoes under "cultivation" by the Indians at Popayan, Pasto, and many other places in Colombia. Any movement of these strange new "roots" from this region and the course that those tubers actually did take that were first introduced into the old world must have been down to the Pacific, north to Panama, across the Isthmus to Nombre de Dios and thence to Cadiz in Spain, unless perchance they were intercepted by buccaneers and carried elsewhere. Owing to the coldness of the Humboldt current these tubers need not have been subjected to a temperature exceeding 24° C. until they reached Panama. Here, and again at sea level on the Atlantic, the temperatures are higher. The data compiled by Reed (42) show a mean maximum temperature for Ancon (Pacific) up to 33.3° C. in March and one for Colon (Atlantic) not

exceeding 29° C. at any time in the year. The data also show that during the months of September, October, and November the mean maximum temperature at Ancon does not exceed 30.5° C. At Ancon the mean minimum temperature ranges from 23° C. downward and at Colon it ranges from 25° C. downward for the several months of the year.

Once out of port the hold temperatures of the small vessels then in use would approximate that of the surface water of the Caribbean. The climatological data of Reed (43) with additional data on surface water temperatures, kindly furnished by the Climatological Division of the United States Weather Bureau, show that at most times in the year this would be about 27° C. and that at no time would it exceed 29° C. In confirmation of this, an official of the United Fruit Company has supplied the information (letter) that the hold temperature of non-refrigerated vessels in Caribbean waters does not exceed 29.3° and rarely is more than 28.3° C. It appears from this that it would have been possible for months at a time to have moved potatoes from the higher Andes or the west coast of South America to European points without subjecting them at any time to a temperature high enough and prolonged enough to have killed any mycelium of *P. infestans* that might have been present. Jensen (27, p. 84) states that mycelium of *P. infestans* in tubers was killed in 65 hours at 30° C. but that it was living after 72 hours at 27.5° C. Jensen's data are by no means corroborated by the recent work of Vowinkle (51), who shows that mycelium grows in the parenchyma of the tuber at temperatures between 3.2° and 30.3° C. At continuous exposure to the latter temperature the mycelium had progressed 4 mm. in 13 days. This leaves a margin of more than a degree between the highest hold temperatures and the thermal death point of the fungus after an incubation of nearly two weeks. The effect of intermittent high temperatures has not been studied except that, in the field, mycelium in leaves and stems occasionally is subjected to temperatures of 32° C. or more for several hours at a time without being destroyed.

The presumption that "wild" potatoes from South America would prove resistant to blight seems to have been in the mind of practically every one who has given much thought to the control of the disease by biological means. The idea of degeneration through continued asexual propagation seems to have been back of the presumption. Goodrich had this idea, and Darwin seems to have had a similar thought. "Darwin's potato," *S. maglia* Molina, was employed in breeding by James Torbitt (Müller, 34) but was finally reported (17) susceptible. Heckel's (22) *S. commersonii* Dunal never has yielded anything except some "sports" about which there has been and still is much controversy. One of the

mutants, however, was reported by Heckel and Verne (23) to be resistant to blight. If the collection of material brought back from South America by André (2, 3) has yielded anything of *Phytophthora*-resisting value, it is not yet evident in the literature nor in the varieties recently listed by French seedsmen. Of the South American material obtained by Verne in 1911, one very encouraging report was made by Heckel and Verne (23) in which *Solanum acaule* Bitter, certain mutants, and the variety Luque amarga from the LaPaz market were recorded as immune. This report was based on the observations of a single year, and no further records seem to have appeared. Müller (34, p. 69), however, has two highly resistant races, one of which ("S.W.") is a hybrid of a South American plant grown by the Chilote Indians with the variety Switez, the other ("Ef") presumably of South American origin but received from a colleague in Berlin without label.

A few "wild" plants have been tested for blight resistance recently at Ithaca, both in greenhouse and in field, but all have been found susceptible. These include three samples of *S. commersonii* (one with a colored tuber, two with white) from the coastal plain of Southeastern South America; *S. maglia* from the island of Chiloé; *S. fendleri* Gray and *S. jamesii* Torrey from Southwestern United States; also a miscellaneous lot of "Indian potatoes" obtained on the market at Cuzco, and comprising perhaps a dozen different sorts.

It can not be denied that Goodrich obtained a resistant plant. His Rough Purple Chili was subjected to severe test through several serious epiphytotics. No stock of this variety is known to exist at present, although Goodrich offered it to the public and it was widely distributed. Its resistance, however, is extant in a number of varieties, and crops out from time to time when a particularly fortunate cross or selfing of these varieties occurs. It is unfortunate that the exact origin of this variety is unknown. Goodrich (20) states that the tubers came originally from Peru, but whether they did or not can never be determined, for it is not known how the original question of their origin was put to the saleswoman in the market at Panama where they were actually obtained. Three other lots of tubers of more certain South American origin were discarded by Goodrich because they were unsuitable for culture and also because they were somewhat susceptible.

The foregoing is simply the development of an idea originally suggested by W. F. Wight of the U. S. Department of Agriculture in personal correspondence. Mr. Wight's idea is that *Phytophthora infestans* is not endemic in South America; else the potato which unquestionably was carried to Europe from there, and not from Roanoke (Wight, 52), would not be so

universally susceptible. In a later letter Mr. Wight suggested that the fungus might have come originally from tomato. The suggestion has much in its favor. The occurrence of blight on tomato is decidedly rare. The only exceptions to this to be noted in North America are two small areas, one in California and one in the Piedmont region of the Alleghanies. When blight occurs in tomato elsewhere it is a matter of special comment. For the most part this seems to be the case in other continents.

At Ithaca, tomatoes of two or three different varieties have been grown very near the potato experimental field in each of three years without showing any infection whatever. A few volunteer tomato plants in the potato rows were inoculated regularly in 1926 but none ever showed infection. It is possible that foliage of this variety is immune to *Phytophthora*, as is that of certain varieties tested by Bondartseva-Monteverde (8). No fruits developed on these plants. Similar negative results have been obtained at times on tomato foliage in greenhouse tests. Whether these were indications of foliage immunity or whether the plants were not left long enough in the moist chamber after inoculation is not known for it was not known until the appearance of Berg's data that this factor was involved. Inoculated potato plants usually have not been held in the moist chamber for more than two hours, and Vowinckle's data as well as our own experience indicate that this is much longer than is necessary when fully matured swarm-spores are used for inoculation on foliage of susceptible varieties of potatoes.

The fact that some varieties of tomatoes show foliage immunity to *Phytophthora* is a substantial support to Wight's suggestion. It is practically certain that tomato breeders have given no thought whatever to *Phytophthora* rot or blight in their work so that the persistence of resistance indicates that this was characteristic of ancestral forms. Wight suggests (letter) that the original home of the tomato is still a matter for investigation. The rôle that the *S. melongenum* may play here has been implied earlier in this paper.

It is not worth while at present to carry speculation too far nor to do more than enumerate some of the difficulties involved. The ancestral type of *S. tuberosum* has not been established with certainty; the name *tuberosum* was clearly applied to a plant in heterozygous condition and unquestionably one subjected to a type of cultivation and even of selection for an unknown number of generations; many or perhaps most of the samples of "wild" *tuberosum* taken to Europe were not of the ancestral type at all but merely escapes; many of the specimens, especially in the earlier years, unquestionably came from irrigated regions where *Phytophthora* could not exist and where the natural selection process could not operate; tracing the

introduction of the commonest plants into new countries is often difficult or impossible—witness the case of the potato itself—and to trace the introduction of inconspicuous Solanaceae often presents even greater difficulties; whether to begin with boreal forms, as the biology of the fungus indicates, is even a question.

Obviously the difficulties are numerous; and clearly for the elucidation of these problems as well as for the confirmation of Vavilov's (49) paradox, the dominance of the recessives, more intensive study must be made of the Solani, especially of the boreal forms as they exist in their native habitat.

SUMMARY

The potato variety Ekishirazu from Japan has remained highly resistant to the attacks of *Phytophthora infestans* at Ithaca, New York, from 1921 to 1927 inclusive, but the variety has no commercial possibilities in New York. Hybrids with various varieties have yielded 46 families of plants possessing the approximate resistance of Ekishirazu, some of them decidedly tolerant to dry weather and some worthy of tests on a commercial scale.

Phytophthora infestans is regarded as exhibiting a low order of parasitism. It is not known to have more than one biologically specialized form.

Phytophthora infestans is not thought to be endemic to South America but to have been introduced there and into Europe on some other solanaceous host.

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OLEANDER BACTERIOSIS IN CALIFORNIA¹

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INTRODUCTION

For the past ten years or more a disease of *Nerium oleander* L. has been found sporadically in the nurseries of California. While the disease has at no time become serious, it has persisted and has occasionally been troublesome to control. The interest in this disease is scientific rather than economic and centers in its resemblances to and differences from other knot and gall diseases, such as olive knot (*Pseudomonas savastanoi* E. F. S.) and crown gall (*Ps. tumefaciens* Sm. and T.). It has been found occasionally in the open, on park and door-yard plantings. The slowness with which the disease has spread may be accounted for by several factors, chief of which are the strict quarantine and inspection of nursery stock for diseases, the unfavorable conditions in many places for the development of this disease, and the rather limited extent to which the oleander is used as an ornamental. At Riverside observations of the disease have extended over eight years. During this time it has persisted, varying in amount according to climatic conditions. The disease in 1926 was especially severe on the seed pods, peduncles, and young leaves. The rainy weather of April and the cool weather of May and June were apparently favorable for a heavy infection.

Considerable confusion has arisen regarding the nature of the oleander disease, largely through the inadequacy of the study thus far given it. It is with the hope of elucidating certain of these disputed points that this disease has been studied again. The results here given are based on data extending over a period of several years of intermittent study. The work has of necessity been largely a comparative study of the three organisms known to cause galls on trees. These three organisms, *Pseudomonas tumefaciens* from peach, *Ps. savastanoi* from olive, and the one from the oleander, have been grown and studied in pure cultures which have been used in making artificial inoculations.

HISTORY AND PREVIOUS STUDIES

Peglion (4) described a tubercle disease of oleander from Italy. He observed certain resemblances to the olive knot and isolated a schizomycete

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from the affected tissue. He made inoculations but published before securing results.

E. F. Smith (8) states: "The organism isolated by Peglion is unlike the olive-tubercle organism, and in Dr. Petri's experiments and in my own, a culture of it having been given me by Dr. Petri, also proved nonpathogenic to the oleander."

C. O. Smith (6) described a bacterial disease of oleanders in California. He cultivated a species of bacteria from the diseased lesions, studied its cultural characteristics in different media, and produced infection with it on the oleander and olive. No artificial inoculations at that time were made on the oleander with the olive-knot organism, then called *Bacillus olea*, but more recently described as *Pseudomonas savastanoi*.

E. F. Smith (8), in his study of the olive-knot organism, artificially inoculated oleanders with it, also making check inoculations on the olive. Negative results were secured with the inoculations on the oleander. He suggests that the oleander tubercle seems to him to be due to *Pseudomonas tumefaciens*. His observations and inoculation experiments (11) led him to think that the oleander disease is not due to the olive-tubercle organism. He also states that this was Petri's opinion.

Smith, Brown, and Townsend (12, p. 31 and pl. 3) also describe and illustrate successful inoculations of the oleander with *Ps. tumefaciens*, and for comparison picture an oleander, having a natural gall, from California. They note that in some instances these artificial galls produced by *Ps. tumefaciens* are very similar in their youngest stages to the young natural galls on oleander received from California. In a footnote (12, p. 32), they state that the organism causing the California disease of oleander is probably not identical with *Ps. tumefaciens*. Artificial inoculations of the olive with *Ps. tumefaciens* gave negative results (12, p. 33, 34, 88).

Savastano (5) concluded a series of artificial inoculations with pure cultures of the olive organism, as well as fragments of olive tubercles, into healthy oleander trees. He states that the bacterial diseases of the olive cannot be transmitted to the oleander.

Evans (2) reports the oleander disease as being of frequent occurrence both in the Transvaal and in Cape Colony. Alcoholic-preserved material of the oleander bacteriosis sent by Evans seemed to be typical of the oleander trouble as found in California, and a microscopic examination of stained sections showed pockets of bacteria in the tissue.

Tonelli (15) describes in some detail the bacteriosis of the oleander in Italy. The disease was found by him in gardens and parks. He isolated a schizomycete from the tumors, made successful inoculations on oleander, and made a limited study of the cultural characteristics. He noted that

negative results were secured when *Chrysanthemum frutescens* was inoculated with the oleander organism, even after five months' time, while positive results were secured with *Ps. tumefaciens* in chrysanthemum and oleander.

Hariot (3) reports the oleander disease in France and refers to the work and views of Tonelli.

The oleander bacteriosis has been reported from Italy, France, South Africa, and California. It probably had its origin in Italy, and was distributed on nursery stock to the other subtropical countries.

SYMPTOMS OF THE DISEASE

The infection on the oleander has been found as aerial galls or lesions on the stems, foliage, and inflorescence (on the peduncles and the ovary, and in artificial inoculations on the petals). It has not been found on the root or on any underground part of the stem, in this respect differing from infection produced by the crown gall organism, *Ps. tumefaciens*, which normally develops on the roots and the underground portion of the trunk. There is considerable variation in the form of the oleander lesions or knots, depending upon the nature of the tissue infected and possibly upon the season of the year in which infection has taken place.

On the older stem tissue the organism develops a more or less spherical knot or gall (Pl. VII, C-D) that may project 1-2 cm. and is 1-2 cm. in diameter. On the less lignified twig the organism forms more of a canker, the hypertrophy taking place within the tissue immediately surrounding the injury or puncture, where it may cause the healthy stem tissue to split longitudinally (Pl. VIII, D).

The knots consist of rather soft or spongy tissue; they are rough, irregular, and at length blackish in color. If the tumor tissue is cut away, small dark-colored areas or pockets (Pl. VIII, F-G) can be seen in the tissue, which show numerous bacteria. These spots are usually found next to the healthy tissue. The natural knots or infections are often found localized in the leaves (Pl. VII, A) or at the nodes of the stem (Pl. VII, C), in this respect agreeing with the olive knot, *Ps. savastanoi*.

On more succulent tissue, the disease is not always localized in a knot, but develops a definite lesion which may spread upward and less often downward in the tissue from the initial inoculation (Pl. VIII, H-J). In this case there is often a distinct channel of infection, recognized externally by a longitudinal swelling of the tissue and often by the appearance of small roundish swellings or secondary smooth tubercles (Pl. VIII, H; Pl. IX, A). The infection on the stem may cause hypertrophy along one side of the branch, thus causing more or less torsion. This hypertrophy often

develops at the extremity of the branch as an enlarged mass of chlorotic tissue or a gall-like canker.

The foliage is variously infected. The disease may be localized at a single point, causing a conical elevation of the tissue, usually toward the upper surface of the leaf (Pl. VIII, A). It may cause a gall very similar to those on the stem but of smaller dimensions (Pl. VII, Ae). Surrounding this hypertrophy is sometimes a yellowing of the tissue as shown in Plate VII, Ab. In an infected leaf, hypertrophies or small secondary tumors are often found on the midrib and on the veins branching from it (Pl. VII, B). The secondary infection in the leaf is carried in the circulatory system of the leaves, possibly in the laticiferous elements that occur in the veins. Young leaves are often infected almost as soon as they unfold from the bud, and show various forms of torsion and curling, depending upon the side on which the hypertrophy has developed. They are often characterized by a marked thickening of leaf tissue and inrolled margins (Pl. VII, A). The midrib often bends toward the stem. When the smaller veins are infected the surface of the leaf is bent toward the upper or lower side, depending upon where the hypertrophy has taken place.

The seed pods become infected in park—and outdoor plantings when the disease is prevalent. This suggests that the organism may be distributed by insects, possibly by bees.

The pistils² are infected in various stages of development, and are diversely distorted (Pl. VII, E-G). If the seed pods have reached some size when infected, they become curved and distorted but produce normal seed. If they are attacked early, as in flower, they become more or less bottle-shaped, are shorter and thicker than normal, and have aborted seeds. The dead petals (Pl. VII, C) remain attached to the infected pistils for a long time after blooming.

CHANNELS OF INFECTION

No tumor strands similar to those which Smith (10, p. 81) has found in his study of crown gall on tobacco have been observed in the study of the oleander knot, but definite channels of infection (Pl. IX, A, E) often develop in the active growing tissue of naturally and artificially inoculated stems and leaves. These infection channels may number from one to several in severely infected tissue (Pl. VIII, H-I). At times the infection from artificial inoculations may become general, and be distributed more uniformly throughout the infected tissue, but even here more or less definite

² The author wishes to acknowledge the courtesy of Dr. Antonio Tonelli, Valsalice (Torino), Italy, for specimens of the European oleander disease, and also for his observation on the infection of seed pods.

lines of infection are usually present, as indicated by the secondary tumefactions.

The channels of infection may originate from a primary inoculation and be arranged in nearly straight lines that often extend through several internodes (Pl. IX, A; Pl. VIII, H-J).

In their early stages of development they are swollen and have a more transparent consistency and a lighter green color than normal tissue. From the channels of infection secondary tumors are developed as small, smooth swellings of the tissue. When their surface is cut away a dark-colored pocket is found which can be traced to a dark-colored strand of tissue usually situated in the cortex of the stem (Pl. VIII, F, G, K). This condition closely resembles the secondary tumors of the olive knot as described by Smith (9, p. 71, 72, and 192; 11, p. 387), who says, "A distinct channel of infection is traceable from the secondary tubercle." These infection channels are found in the cortex of the oleander and are apparently distinct from the vascular system, but may possibly in their earlier stages be associated with the laticiferous vessels.

Ps. tumefaciens, when inoculated into oleander, has a tendency to form secondary tumors (Pl. IX, J) which are situated near the primary inoculation. No typical tumor strands (metastases) have been observed in the inoculation of oleander with *Ps. tumefaciens*.

In the leaves the channels of infection apparently follow the veins but are apparently distinct from the vascular system, as cross-sections of veins and petioles fail to show the organism present in the vessels, while masses of bacteria are readily found in the parenchyma. It is believed, but not with certainty demonstrated, that the organism may use the laticiferous vessels which are found in the veins.

The oleander knot often spreads by infection channels from the stem to the leaf tissue and vice versa. It may spread from an inoculation in the midrib to the secondary veins (Pl. VII, B).

ARTIFICIAL INOCULATIONS

The oleander bacteriosis organism has been successfully inoculated into oleander, *Nerium oleander* (Pl. VIII, D-K), the olive, *Olea europea* Linn. (Pl. VIII, C), and certain other hosts related to the olive, as *Adelia* (*Forestiera*) *acuminata* Michx. (Pl. VIII, B) and *Chionanthus virginica* L. (Pl. VIII, A). Negative results were secured with the oleander organism on *Fraxinus floribunda* (S. P. I. 47687, United States Department of Agriculture, Bureau of Seed and Plant Introduction), *F. velutina* Torr., *Osmanthus ilicifolia* Lieb., *Osmanthus fragrans* Lour., *Schinus molle* L., *Prunus salicina* Lindl. \times *P. simonii* (Wickson plum), *P. cerasifera* Ehrh., *P. simonii*

Carr., *P. eriogyna* Mason (synonymous with *P. fremonti* S. Wats.), *Amygdalus persica* L., *A. communis* L., *Vinca*, *Coprosma baueri* Endl., *Thevetia nereifolia* Juss., *Carissa grandiflora* DC., and *Chrysanthemum frutescens* L. The above species of *Prunus* are especially susceptible to the crown-gall organism.

On *Thevetia nereifolia*, the area about the puncture was dark in color and contained a limited amount of dead tissue, but no typical lesions developed. Small hypertrophies are believed to have been produced (only once) on a species of privet, these being little more than knobs on the margin of the healing tissue.

The oleander bacteriosis is readily reproduced artificially in oleander by puncture inoculations as recorded by C. O. Smith (6) and Tonelli (15). Repeated successful artificial infections on both olive and oleander have been made from pure cultures or with diseased oleander tissue. The artificially produced hypertrophies on the olive (Pl. VIII, C), however, differ from those on the oleander (Pl. VIII, E) in being more nearly spherical, or sometimes irregular, elevated growths which enlarge rather rapidly into simple or multiple gall-like tumors consisting of soft tissue extending beyond the stem for 2 to 3 or more centimeters. This abnormal tissue remains green for several months with but little necrosis. During the second year little new development of the knots takes place, and at the end of the season the tissue of the twig is dry and dead as far back as the normal healthy tissue. It is thought that these galls are much shorter-lived than the true olive knot caused by *Pseudomonas savastanoi*. No metastasis, secondary tumors, or channels of infection have been observed in the artificial inoculations of the olive with the oleander organism.

These artificial galls (Pl. VIII, C) on olive produced by the oleander organism have certain resemblances to the olive knot (Pl. VII, H) and to the galls produced on other hosts by *Pseudomonas tumefaciens*, although apparently differing somewhat from both. The olive knot due to *Ps. savastanoi* is usually more regular in shape and has a smoother surface and a wider base of attachment than the oleander knot on olive. *Ps. tumefaciens* apparently does not infect the olive. This statement is based on the negative results from large numbers of artificial inoculations with a strain of *Ps. tumefaciens* isolated from *Prunus* (peach, almond, and plum). The virulence of this strain of *Ps. tumefaciens* was tested by other positive inoculations made at the same time and place on different kinds of *Prunus*. Smith, Brown, and Townsend (12) record several unsuccessful attempts to inoculate the olive with *Ps. tumefaciens* isolated from Paris daisy, peach, and hop. In an investigation by Smith and Quirk (14, p. 505) it is shown that young olive shoots have a highly acid juice (pH 5.20 to 5.28), while the acid

limit of *Ps. tumefaciens* is only about pH 5.70 in peptone beef bouillon. The acidity of the olive is probably the chief factor making it resistant to *Ps. tumefaciens*. It will be noted that *Ps. savastanoi* and the oleander organism have a somewhat lower pH limit (about pH 5.3 in peptone beef bouillon) than *Ps. tumefaciens*.

The oleander organism artificially inoculated into the oleander usually causes slight hypertrophies to develop around the puncture and within the wounded tissue, often causing it to split apart by the abnormal growth. In young, rapidly growing tissue the infection causes the tissue to become swollen and to take on a clear, lighter green color, often with a water soaked appearance near the outer edge of the margin.

A darkening of the surface and the interior tissue surrounding the lesion soon takes place, and is to be found beneath both the secondary infections and the primary lesions, often extending in the latter case into the pith. Within this abnormal tissue and near the healthy tissue may often be found dark-colored pockets that contain numerous bacteria (Pl. VIII, F, G). The lesions that are externally evident are often rather large in size, but when inoculated tissue that to external appearance is apparently normal is cut into it may show blackened necrotic regions. The inoculation of oleander with *Ps. tumefaciens* always produces small galls (Pl. IX, J) rather than lesions or cankers.

The young pistils of the open flowers are readily infected by atomizing with a suspension of the oleander organism (Pl. VII, F). A few typical hypertrophies have also appeared on the inoculated petals.

The inoculations on *Chionanthus virginica*, white fringe, produce definite but usually not typical knots. They show around the punctures at first as an abnormal swelling, 2 to 3 millimeters in diameter, which is of a darker color and has a more or less water-soaked appearance. Some further inoculations made on new growth of *Chionanthus virginica* gave spherical knots (Pl. VIII, A) which were more like olive knot and somewhat similar to those produced by the oleander organism on olive. Sometimes cylindrical, point-like outgrowths develop on the same twig with the more spherical knots. In the inoculations of June 15 (see table 1) peculiar abnormal cylindrical growths appeared both on the stems (Pl. VIII, Ae) and on the leaf petioles (Pl. VIII, Ad).

The inoculations on *Adelia* gave spherical galls that were somewhat similar to those made by the oleander organism on olive. Dilution cultures from these knots as well as those of *Chionanthus* produced numerous colonies in petri plates. When transfers from these were inoculated into oleander, typical lesions and knots developed.

TABLE 1.—*The results of inoculating four different hosts with the oleander disease organism.*

Host inoculated	Date of inoculation, 1920	No. punctures made at inoculation	Knots produced	
			No.	Diameter (in mm.)
<i>Chionanthus virginica</i>	Apr. 4	10	1	5
	Apr. 10	10	7	5
	June 12	10	9	3
	June 15	20	6	6
<i>Olea europea</i>	Apr. 4	10	3	3
	Apr. 30	10	4	5
	June 12	10	5	12
	June 15	10	5	5
	June 23	20	14	5
<i>Adelia acuminata</i>	Apr. 30	10	6	3
	June 12	10	4	6
<i>Nerium oleander</i>	Apr. 4	10	0	—
	Apr. 30	10	6	3
	June 12	10	7	6
	June 15	10	5	5

PATHOLOGICAL HISTOLOGY

The presence of bacteria in the hypertrophic and hyperplastic tissue which had been produced by inoculation with the oleander organism was readily demonstrated by employing the usual histological methods. The tissue was killed in Carnoy solution and sectioned with a sliding microtome, or, where suitable, with a rotary one, from material embedded in paraffin. Acid fuchsin was used as the stain.

Infected tissue of *Nerium oleander* (oleander), *Olea europea* (olive), *Chionanthus virginica* (white fringe), and *Adelia (Forestiera) acuminata* was sectioned and studied. In these studies, tissue masses of different sizes were selected that had been artificially infected for various lengths of time. In the infected tissue when sectioned, a dark-colored area usually marked the location of the infected areas and channel of infection. This was especially true of the oleander, where these areas were found in the cortex, pith, or leaves.

A study of oleander tissue, showing infection channels and secondary tumors, was made from young, vigorously growing tissue, inoculated June 17 and fixed Oct. 27 (Pl. IX, E). A cross section of the affected area showed, in the cortex, small dark-colored circular or irregular areas, 1 milli-

meter or less in diameter (Pl. IX, F). A microscopic examination showed definite bacterial pockets. In the center were broken-down tissue and masses of the causal organism (Pl. IX, G). Surrounding the bacterial masses and disorganized tissue were several layers of small, thin-walled cuboidal cells. Around these were layers of larger, more or less flattened cells, which in turn were surrounded by normal cortical tissue.

Very young, rapidly growing tissue of oleander may develop a visible infection strand in from nine to forty days. Such material inoculated November 5 and fixed December 9 was imbedded in paraffin and sectioned (Pl. IX, Ae). Here a definite infection strand is shown extending between two primary inoculations, and through two internodes and then into the petiole of the leaf. Definite lesions developed at the points of inoculation but no typical galls.

In cross sections the tissue was seen to be enlarged on the side where the infection strand was visible. Five areas in the broken-down cortical tissue showed masses of bacteria indicated by x in Plate IX, B. At these points an increase in number of cells, but not in size resulted, and only parenchymatous tissue was formed (Pl. IX, C). The vessels adjacent were rarely found to be infected.

In another series of inoculations on oleander at the same time and place with a different culture, a similar infection strand developed in nine days. Longitudinal sections showed bacteria in masses occupying definite rows in the tissue. Two or more of these narrow bacterial masses may be parallel to each other as in Plate IX, D, where the organism may possibly have been distributed through the laticiferous vessels. This, however, requires further demonstration.

An inoculation of the midrib of an oleander leaf will often result in the infection developing through the entire vascular system of the leaf (Pl. VII, B). The bacterial pockets seem to have no definite location in the midrib. Sometimes a large mass of bacteria is at the center, while in other sections several smaller masses are situated in the parenchyma near the outside of the midrib. Similar masses of bacteria were found in the parenchyma of an infected petiole of oleander.

Sections of galls produced on the olive and on *Adelia* (*Forestiera*) *acuminata* by the oleander organism also showed masses of bacteria, often associated with necrosis of tissue. These pockets were sometimes located in the center of the gall and at other times in other parts of the enlarged tissue. The hypertrophies were for the most part parenchymatous, with vascular elements arranged in an irregular way. In a gall on olive, 15 millimeters in diameter, caused by the oleander organism, three dark-colored lines were found extending through nearly to its base, where they terminated in a

dark-colored center, an area in which numerous bacteria were found in mass (Pl. IX, I).

The infection on stems of *Chionanthus virginica* may develop into a peculiar branching growth 6 to 7 millimeters in length (Pl. VII, Ae). At each of the tips of this growth was a groove-like depression that seemed to divide in into two symmetrical parts. A cross section of these galls showed an interesting arrangement of tissue (Pl. IX, H). On the outside was a normal layer of bark epidermis, and within this was a layer several cells wide consisting of large and thin walled cells, that did not stain deeply with acid fuchsin. Next within this tissue was an area of smaller cells also not staining deeply. In the center of the latter tissue were two cavities evidently corresponding to the two branchings of the outgrowth. These cavities contained large masses of bacteria, which could be observed at the margin of the cavity where they had not been washed out in treatment. There was apparently no necrosis of the tissue in these knots. Some vascular tissue (spiral vessels) was found immediately surrounding the center cavity. Binucleated cells were occasionally found in the outer zone of this tissue. Galls similar to those on the stems were produced on the petioles of leaves of this host (Pl. VIII, Ad). The tissue showed an arrangement similar to that just described but with more vascular tissue present.

Sections of tissue infected with the oleander organism rarely failed to show numerous bacteria associated with broken-down tissue. This is in marked contrast to the absence of organisms as observed by Smith, Brown, and McCulloch (13) in their study of the structure of galls on hosts infected artificially by *Ps. tumefaciens*.

RELATIONSHIP OF CAUSAL ORGANISM

The artificial inoculations and the etiological and cultural characteristics indicate that the oleander organism is more closely related to *P. savastanoi* than to *P. tumefaciens*. Repeated inoculations with *P. savastanoi* on *Nerium oleander* have always given negative results, thus agreeing with the work of Smith (8) and Savastano (5), but the olive-knot organism, according to C. O. Smith (7), will infect certain hosts related to the olive, as *Adelia* (*Forestiera*) *acuminata*, *Chionanthus virginica*, *Osmanthus aquifolia*, *Jasminum primulinum*, *Fraxinus velutina*, and *F. floribunda*. The positive results of inoculation of the oleander organism on olive *Olea europea*, *Chionanthus virginica*, and *Adelia* (*Forestiera*) *acuminata*, and the negative results on species of *Prunus*, sugar beet, *Beta vulgaris*, *Thevetia*, and *Carissa* are significant in pointing to its relationship to *P. savastanoi*.

The careful work done by Smith, Brown, and Townsend (12) in studying various strains of *P. tumefaciens* has shown variations in cultural char-

acteristics of these organisms that are difficult to explain unless they are regarded as different strains or races or perhaps species.

The oleander and olive organisms have characteristics not differing so very much from certain of these strains, but they could hardly be regarded as belonging to the tumefaciens group. If the oleander organism is described as a new species, the following facts should be considered: (1) The olive organism *Ps. savastanoi* is not parasitic on the oleander, and cannot be made to cause knot on it; (2) the knots produced by the oleander organism on the olive differ somewhat from olive knots; (3) the cultural and morphological characteristics of the two are not sufficiently different to separate the two into different species; (4) the oleander strain often develops infection channels in the oleander which are very similar to those formed by the olive organism in the olive; (5) the two are not distinguishable morphologically. While the resemblances between the olive and oleander organisms are close and the differences difficult to detect, still these organisms are probably different strains although not distinct species. E. F. Smith (11, p. 404) believes that there are very likely several strains of *Ps. savastanoi*, all pathogenic but somewhat different. He describes (11) an ash disease from Europe that is very similar morphologically and culturally to the olive tubercle, and yet not absolutely identical. It is caused by an organism pathogenic on the ash, *Fraxinus excelsior*, but not on the olive, and this organism is regarded as a variety of *Ps. savastanoi* rather than as a different species. It is of interest to note that C. O. Smith (7) produced knots on *Fraxinus* artificially with *Ps. savastanoi* but not with the oleander organism, yet the latter did make knots on other hosts in addition to the olive and oleander. It seems best with our present knowledge to regard the oleander organism as being a variety or strain of *Ps. savastanoi* rather than to make a new species. The name *Ps. savastanoi* var. *nerii* is suggested.

Cultural Characteristics

Pseudomonas savastanoi var. *nerii* n. var.

Rods with rounded end, $1.5-2.5 \times 0.5-0.6 \mu$; spores and capsules absent, flagellae polar, 1-3; colonies after 8 days on potato dextrose agar at 25° C., 1-3 millimeters in diameter, flat, circular, shining, often with a somewhat undulate margin. Young colonies with a bluish cast by transmitted light, carbon gray³ by transmitted light, reticulations sometimes present especially near margin, which is of a paler (nearly hyaline) color, flaky white particles sometimes present, but transitory. Colonies after 8 days on gelatin smaller, 1-2 millimeters in diameter. Gelatin not liquefied or stained. Ex-

³ Robert Ridgway. Color standards and color nomenclature, Washington, D. C., 1912.

cellent growth in nutrient beef agar; better growth in potato dextrose agar, spread out in a rather thin layer, often with an irregular lobed margin, shining, opalescent, changing to pale gray in 10 days. On litmus peptone dextrose agar slants, the litmus promptly (48 hours) and permanently changes to a bright red. Growth at first vigorous, but limited in amount and soon ceases. The same reaction takes place in litmus peptone galactose agar. In litmus peptone saccharose agar some purpling of medium near growth, deep vinaceous by reflected light.

In liquid medium, nutrient beef bouillon, showed dense cloudiness with a partial pellicle and small amounts of sediment. In beef bouillon under chloroform, unrestrained growth. In 2 per cent peptone no pellicle or precipitate formed, color becoming almost a honey yellow (100 days). Slight cloudiness in 2 per cent saccharose without pellicle or precipitate (35 days). In tubes autoclaved 15 minutes at 15 pounds pressure, slight cloudiness without ring or pellicle after 11 days, but densely clouded after 20 days. In maltose peptone cloudiness developed without pellicle or precipitate (10 days), becoming somewhat browned (100 days). Saccharose 2 per cent, peptone 1 per cent, produced a partial pellicle and ring formation, moderately clouded, somewhat browned in color.

No gas was produced in fermentation tubes (20 days) containing 2 per cent peptone and 1 per cent of the following carbon compounds: dextrose, galactose, lactose, saccharose, maltose, mannite, and glycerin. The liquid clouded promptly (5 days) and was confined to open arm. Litmus milk gradually blued, strong alkaline reaction (5-10 days). No whey or separation of casein, reduction of litmus slow, tendency for it to clear (2-3 months). Cultures continue to darken after inoculation; one series changed from lavender to pale vinaceous and pale Valey's gray (10 days), plumbago blue (25 days), slate and slate purple (90 days). The liquid at this time is one half of former volume but shows no whey. Methylene blue shows some fading of color (3 days); pale methylene blue (7 days), becoming tea green (14 days), and sage green and American green (4 months). Milk gave no separation of casein; white color gradually disappears, becoming ochraceous buff (15 days), pale ecru Dauthenay (1, p. 166, No. 4) (28 days); buff Dauthenay (1, p. 209) and in some tubes dark brown Dauthenay (1, p. 304) (4 months). At this time tubes had the appearance of clearing, but were not entirely so even after 6 months. Cohn solution gave cloudiness sometimes with and at other times without crystals. When transferred to Cohn solution from tubes of bouillon, the organism sometimes failed to grow; while good growth resulted when an agar slant was used. No growth on Uschinsky's solution. A positive indol test in 1 per cent peptone (Wittes) and distilled water, using the sodium nitrite

test. Standing or heating in hot-water bath was necessary to produce the pink color. Thermal death point is 51° C.

The pH limits of growth in peptone meat extract bouillon for *Ps. savastanoi* and *Ps. savastanoi* var. *nerii* are the same, being pH 5.3 to pH 8.9 (Colorimetric method).

SUMMARY AND GENERAL DISCUSSION

1. The oleander organism was grown on the same culture media as that of the olive knot, *Pseudomonas savastanoi*, and showed practically identical cultural characteristics. The differences are very slight and are pointed out in the descriptions of the oleander organism.

2. *Pseudomonas tumefaciens* (isolated from peach) differs markedly from the olive and oleander organisms in cultural characteristics, pathogenicity, and reaction on hosts. It is more vigorous in growth than those organisms and is apparently distinct from them.

3. In liquid media the olive and oleander organisms develop a fine cloudiness held in suspension and at times a partial or very thin complete pellicle, but sometimes only a ring-like formation. The following liquid media were used in its growth: beef bouillon, 2 per cent peptone solution, 2 per cent saccharose, 2 per cent dextrose, dextrose peptone solution, saccharose peptone, and maltose peptone solutions, and asparagin in solution.

4. *Pseudomonas tumefaciens* grew on the above liquid media in a very different manner, producing almost no cloudiness, but a growth of floccules and threads which formed a definite precipitate, or remained in suspension in the nearly clear liquid. A pellicle was almost always present and was often dense. Growth occurred in saccharose and saccharose peptone solutions, with inversion of this sugar.

5. The reaction of the olive and oleander in milk and litmus milk was very different from that of *Pseudomonas tumefaciens* (peach strain). The latter separated the casein after several days and reduced the litmus, at length developing a brown-colored liquid coagulum, while the other two organisms developed shades of blue and were always without a coagulum or whey. It is of interest to note that according to Smith, Brown, and Townsend (12, p. 154) certain strains of *Ps. tumefaciens* react in litmus milk very similarly to the oleander and olive organisms.

6. The reactions and growth on solid agars differ. *Pseudomonas tumefaciens* grows along the stroke in a piled-up manner. The other two have a flatter, more spread-out, less watery-appearing growth. The growth of *Ps. tumefaciens* on dextrose and galactose peptone litmus agars does not redden the litmus like the oleander and olive organisms, and its growth is unrestrained in this medium.

7. The oleander organism produces more or less evident galls on some hosts, while on others lesions are formed with hypertrophies within or at the margin of the healing tissue. The organism is found in masses in spaces occupied by broken-down tissue but not within the cell.

8. The oleander organism was pathogenic on the olive and certain hosts that are botanically related to the olive. It was pathogenic on the oleander (its natural host), but not on certain hosts that are related to the oleander. Some of the same hosts susceptible to the oleander organism were also susceptible to the olive knot organism when artificially inoculated. The oleander organism was pathogenic on olive (*Olea europea*), *Chionanthus*, *Adelia*, and *Nerium oleander*. The olive knot organism was pathogenic on the same hosts, with the exception of oleander and the addition of species of *Fraxinus* and *Osmanthus*. Negative results were secured with the olive and oleander organisms on species of *Prunus*, on *Chrysanthemum frutescens*, *Carissa grandiflora*, *Vinca* and *Thevetia nereifolia*.

9. The olive and oleander galls produced by the oleander organism showed definite pockets or masses of the causal organism. These in the oleander were readily detected with the unaided eye as small black areas in the greener, hypertrophied tissue. The same necrotic tissue was found when secondary tumors and infection strands were sectioned.

10. The characteristics of the oleander organism were not sufficiently distinct from those of the olive-knot organism to justify making it a distinct species. The pathogenicity was very similar except that the olive organism did not infect the oleander. For the present it seems best to regard the oleander organism as a variety of the olive-knot organism. If a name is desirable, *nerii* is suggested (*Pseudomonas savastanoi* variety *nerii* n. var.).

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EXPLANATION OF PLATES

PLATE VII

Oleander tissue except in H. A: Natural infection on leaves; light areas indicate yellowing of tissue. Observe rolling of young leaves and conical infection on margins. B: Artificial inoculation in midvein and subsequent invasion of vascular system. C and D: Oleander stem from a park planting. Note at upper left the infected pistils with petals still attached. E: Diseased oleander seed pods sent from Italy by Dr. Antonio Tonelli. G: California infected oleander seed pods.

H. Natural knots on olive caused by *Ps. savastanoi* for comparison with those on olive by oleander organism (Plate VIII, C).

PLATE VIII

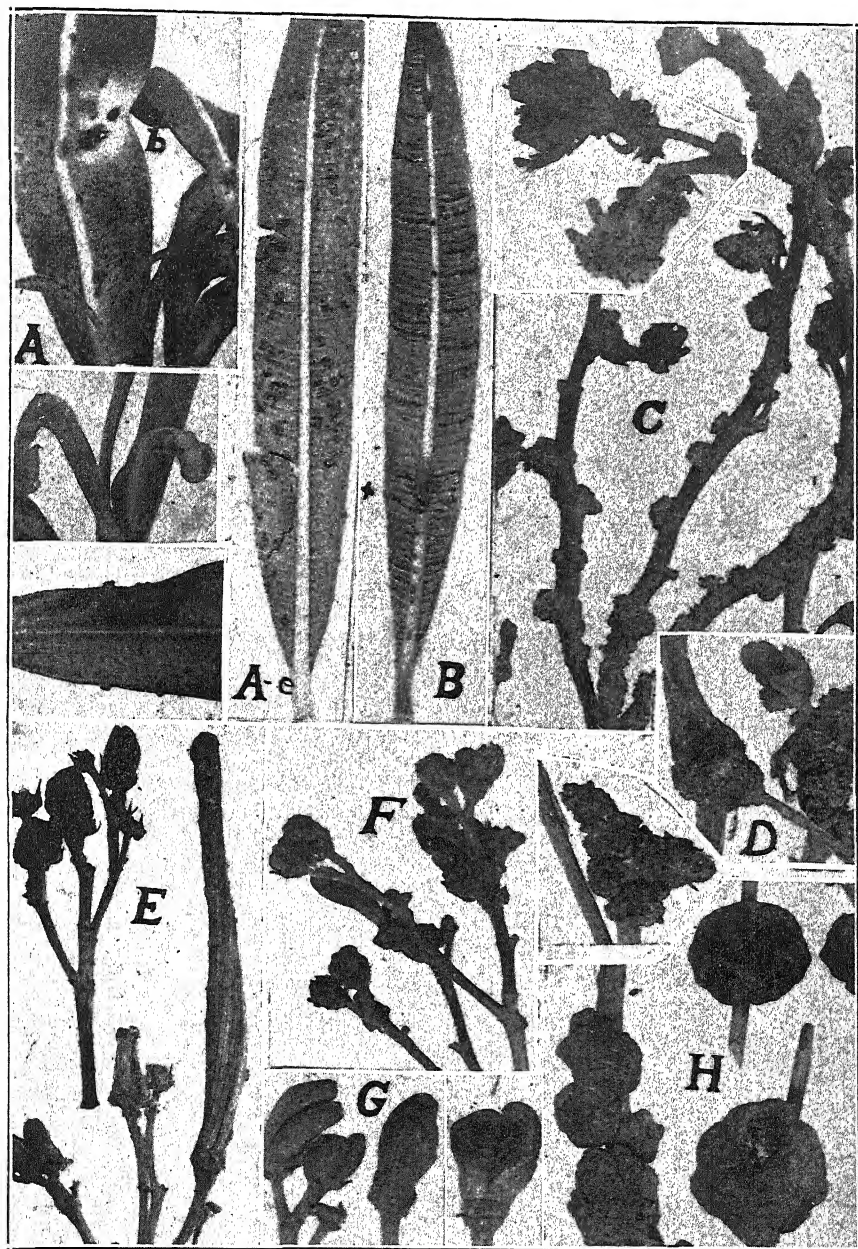
Artificial inoculations with oleander organism. A: Knots formed on white fringe (*Chionanthus virginica*) after three months. Peculiar outgrowths are shown on leaf petiole and on two twigs at right. For cross section of e see plate IX, H. B: Knots on *Adelia (Forestiera) acuminata* after three months. C: Knots on olive after five months. D: Splitting of oleander tissue by growth within. Photographed six months after inoculation. E: Knots on oleander after 35 days. F and G: Surface of J cut away revealing the darkened interior tissue and bacterial pockets. H: Secondary lesions formed on oleander between primary inoculations at x. I: Elongated secondary tumefactions on oleander. J: Channel of infection on oleander that developed from positive artificial lesions at x. K: Infected cortical oleander tissue.

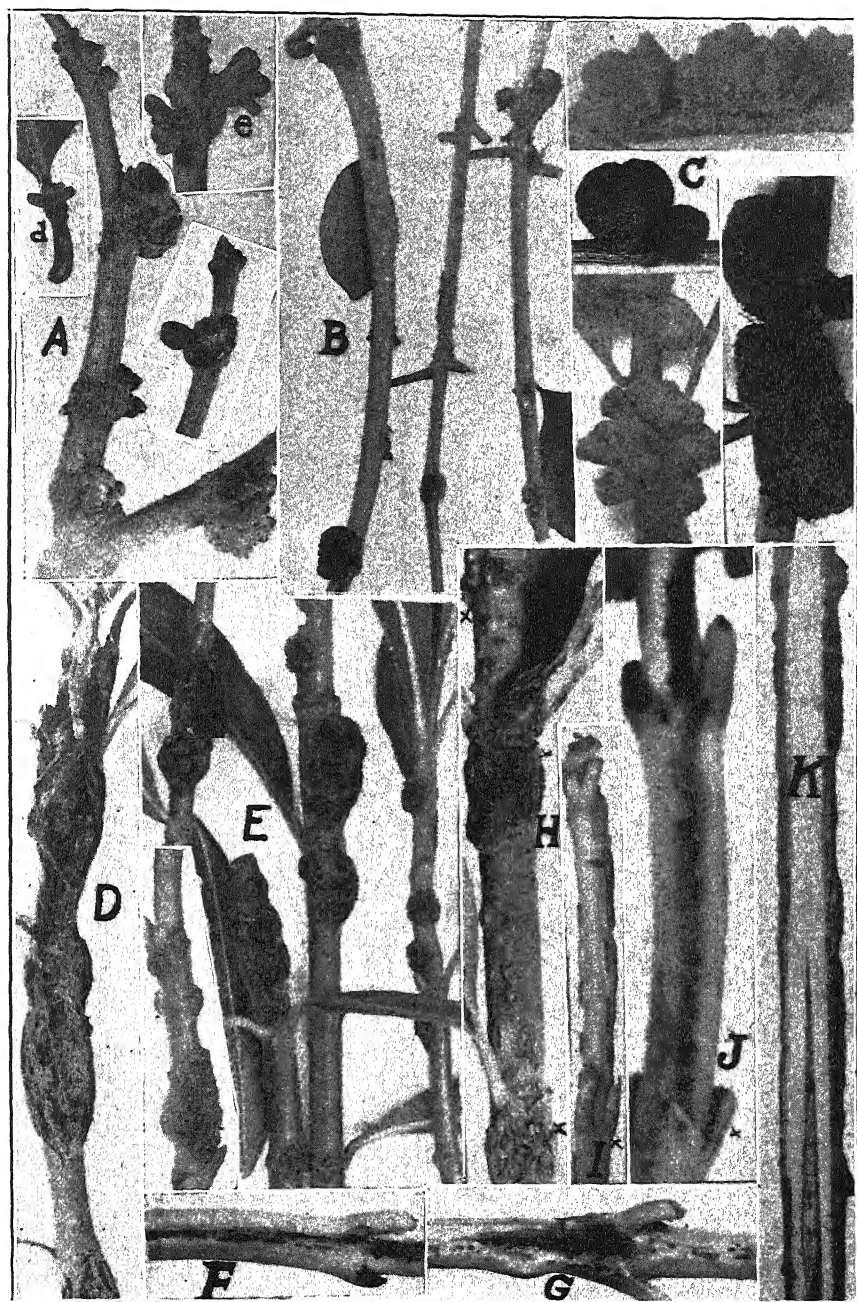
PLATE IX

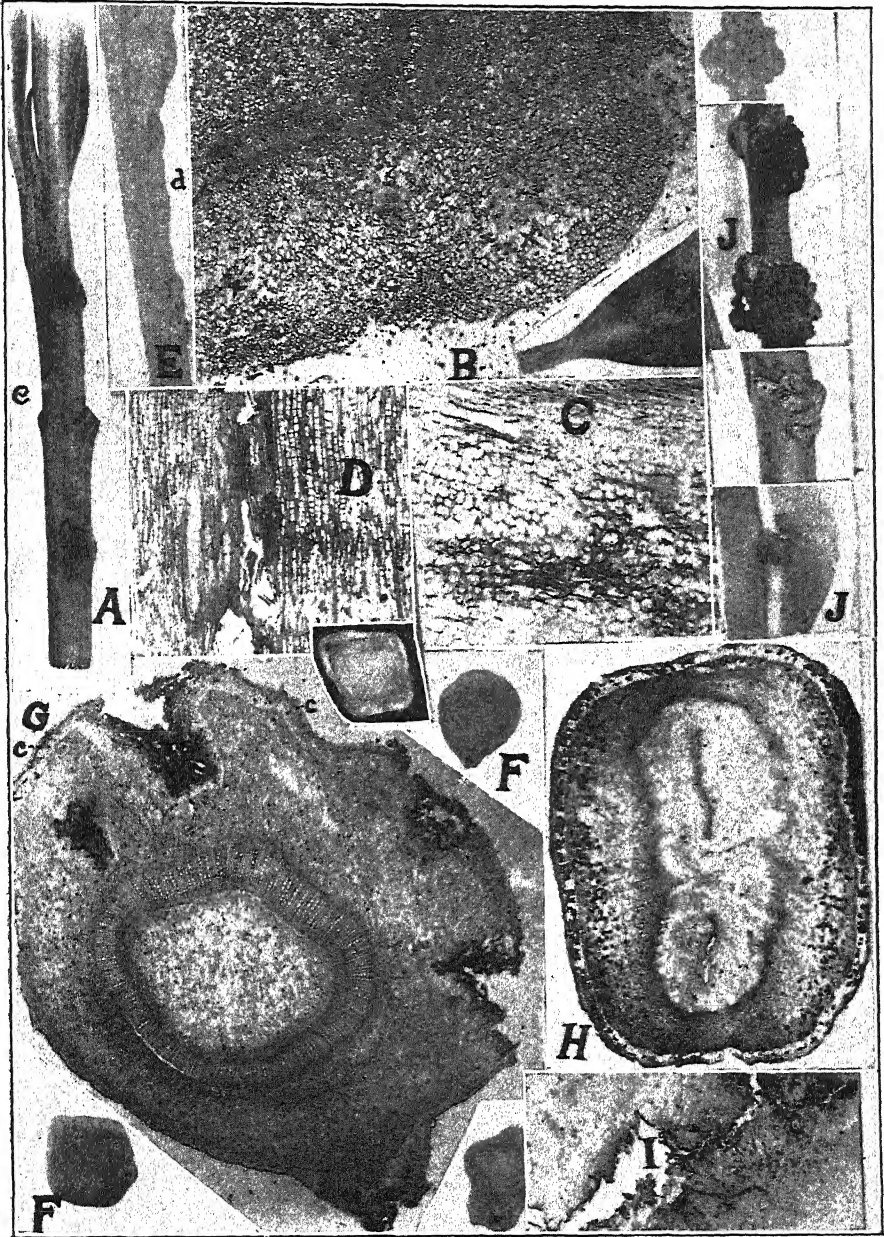
Artificial inoculations and sections of diseased tissues. A: Two primary inoculations on oleander and infection channel connecting them and extending into the

leaf petiole; photographed 35 days after inoculation. B and C: Cross and longitudinal sections of stem in A; made above the node at e; note in B the five bacterial areas at x, and the broken-down tissue in C. D: Longitudinal section through areas of bacteria in an infection channel similar to A from an inoculation made at the same time with a different culture. E: An older infection channel through which sections were made at d showing blackened areas as seen in F. G: A cross-section of F magnified to show bacterial areas in cortex. Other initial bacterial areas are shown at G, e.

H: Section of artificially produced knot in *Chionanthus* (Plate VIII, A, e). I: Section of an artificial knot of olive produced by the oleander organism. J: Infection on oleander with *Ps. tumefaciens*. Note above upper gall two small tumefactions.







DRY ROT OF GLADIOLUS CORMS

L. M. MASSEY

The term "dry rot" was first used by Wallace (9), who recognized four diseases of gladiolus corms, namely, hard rot, dry rot, soft rot, and scab. Although Wallace states (9, p. 69) that he was probably including two distinct diseases under the name "dry rot," his brief description of one of the two fungi, isolated from corms from Germany and New York State, leaves no doubt as to the identity of the disease with the one here described. With the termination of the study by Wallace, Fitzpatrick (3) gave some attention to gladiolus diseases and throughout his study restricted the term dry rot to the disease as it is now known. The writer (4) adopted the terminology of the above-mentioned workers, as did Drayton (2) in presenting the most extensive account of the disease thus far published.

Although mention of gladiolus diseases is to be found in several publications antedating the articles cited here, the meager descriptions of both disease and pathogene and the absence of infection experiments create doubt as to what diseases were involved. Wallace (9, p. 14) states that "the disease reported by Robinson (8, p. 139) is probably identical with that which we are calling dry rot, specimens of which were received from Germany and also from New York State. At least the presence of a fungus with *Rhizoctonia*-like mycelia in the tissue suggests this." Aside from this doubtful instance the dry rot disease can not be identified in literature prior to 1909.

Dry rot probably occurs generally wherever gladioli are grown. Hundreds of specimens have been received from growers in the United States and Canada and the disease has been found on corms from England, France, and Holland. Dry rot, hard rot (5), and neck rot (7) are the most common and prevalent diseases of the gladiolus, a plant which has greatly increased in popular favor and economic importance during the past two decades. The losses from these three diseases are large, especially in years of high precipitation and when there is unfavorable weather at the time of harvesting the corms.

SYMPTOMATOLOGY

All parts of the plant below ground may be infected, and a stem-rot at the surface of the soil involving the lower inch or two of the stem may

often be observed. This rotting of the stem frequently results in a premature yellowing of the tops, followed by drying and death. The plant may or may not bend at the rotted area, depending on the extent of decay, which in turn is dependent on the amount of moisture present. Usually in cases of stem-rot the corm will be found to be affected, but instances may be observed in which the corm appears quite free from lesions. It is possible that in these cases the husks or sheathing leaf bases of the parent corm are diseased. In stem-rot the outer sheathing leaf bases are characteristically attacked first. Minute, black sclerotia of the pathogene are usually present on diseased stems.

As in the case of hard rot (5, p. 157), but less frequently, yellowing of the foliage and death of the plant may result from the decay of the parent corm affected with dry rot when planted. This usually happens about mid-season when temperatures are high and the soil dry. Saprophytic fungi are frequently involved in the final destruction of the old corms, although the dry rot advances more rapidly under the favorable environment of the soil and may of itself cause the death of the plants.

The dry sheathing leaf bases or "husks" of stored corms affected with dry rot may show evidences of disease by being abnormally dark in color and brittle. These darkened areas are usually irregular in outline, rarely being approximately circular as are the lesions on the corms. There is a tendency for the affected husks to split longitudinally, and pieces may be broken away easily, exposing the corm beneath (Plate X, B). The husk lesions may or may not be immediately over lesions on the corms. In most cases it is necessary to remove the husks to determine whether or not a corm is diseased.

On removing the husks it will be found that diseased corms characteristically bear many small lesions which range in size from mere dots to areas about one centimeter in diameter (Plate X). Larger individual areas are relatively uncommon, but are to be found and may involve half or more of the corm. Frequently, numerous lesions coalesce to form a large lesion, which in advanced cases may involve the entire corm and reduce it to a dry and shriveled mummy. When this condition exists it is usually still possible to trace the outlines of individual lesions. Areas more or less healthy may be left insulated in large areas of diseased tissue. Not uncommonly the lesions occur along the juncture of the husk and the corm, forming rings of diseased tissue. This would seem to indicate that the husk was first affected, the rot spreading from the husk to the corm. On the other hand, diseased corms with apparently healthy husks are commonly found.

The individual lesions are approximately circular in outline and appear first as minute, reddish brown spots, usually on the side and lower half of the corm but not infrequently on the upper half as well. The line of

demarcation between healthy and diseased tissue is rather sharp. As the lesions increase in size, the centers become sunken, the color usually deepens to black, and the margins become more definite. The sunken centers of the lesions probably result from the drying of the tissue. The margins of the lesions, especially noticeable in the larger and older diseased areas, are slightly elevated. The consistency of diseased tissue is characteristically corky.

As a rule, the lesions do not extend deeply into the corm, the range being from 1 to 5 or 6 millimeters and the average less than 3 millimeters. Usually the diseased tissue can be separated readily from the apparently healthy tissue beneath. This symptom is not restricted to the dry rot disease but is at least equally true of hard rot, *Fusarium* rot, and scab. Evidently, in this stage of the advancement of the disease, the corm has the advantage in being able to callous over the diseased area and, for a time at least, prevent further advance of the invading hyphae.

A more detailed study of the larger and older lesions will sometimes disclose the presence of minute black bodies buried in the diseased tissues. These are the sclerotia of the pathogene. They are sometimes present on the husks (Plate X, E) but are not found in a sufficiently high percentage of instances to be of much importance as an aid in diagnosis.

Cormels produced by diseased plants are commonly affected. At the time of harvesting the corms it is frequently possible to distinguish diseased cormels by their darker color, but this difference in color is less marked on drying, if at all evident. The lesions to be found by removing the hard outer coats are similar to those found on the corms, and mummification during storage results in a high percentage of cases.

ETIOLOGY

The dry rot of gladiolus is caused by the fungus *Sclerotium gladioli* n. sp.

The pathogene is readily obtained in culture by the usual methods of making isolations from infected tissue. It is a relatively rapid grower and will tolerate a wide range of media and cultural conditions. As stated below, growth varies somewhat with different media, but the variations are not great enough to render difficult the identification of the fungus.

Sclerotia similar to those produced on stems and husks, and sometimes within the infected tissues of the corms, are readily formed in culture in from 10 to 14 days (Plate XI). Measurements of 100 sclerotia produced on potato agar¹ in a petri dish after three weeks at about 20° C. gave a diameter

¹ In preparing the potato agar 200 grams of potato were used per litre, to which were added 20 grams of agar. For cornmeal agar, 50 grams of cornmeal were used per litre, with 2 per cent agar. Media did not contain sugar unless indicated.

range of $90\text{--}300 \times 90\text{--}240 \mu$, with an average of $191 \times 164 \mu$; on potato agar in a petri dish, 27 days old, at about 20°C ., a range of $125\text{--}261 \times 125\text{--}208 \mu$, with an average of $188 \times 161 \mu$; on cornmeal agar¹ in a petri dish, 27 days old, at about 20°C ., a range of $115\text{--}229 \times 105\text{--}208 \mu$, with an average of $182 \times 159 \mu$; and for 100 sclerotia formed on the stem of a diseased plant (natural infection) growing in the greenhouse, measurements showed a range of $94\text{--}156 \times 73\text{--}135 \mu$, with an average of $119 \times 106 \mu$. Drayton (2, p. 206) gives the range as 187×156 to 93×93 with an average of $158 \times 118 \mu$, the source of the sclerotia not being given. Evidently they vary somewhat, depending on conditions under which they are formed.

The sclerotium is black. It consists of a definite cortex of thick-walled cells, from one to two cells in width, surrounding thin-walled parenchymous cells (Fig. 1, A). On crushing a sclerotium, small globules of an oily substance are exuded. Although growths are obtained from planting sclerotia on artificial media in petri dishes, there is no evidence that the sclerotia, and not attached or adhering hyphae, initiate the culture.

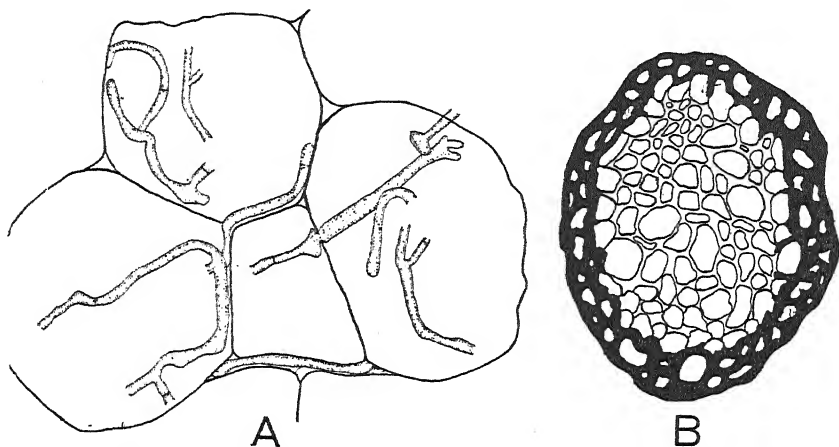


FIG. 1. A, Mycelium is inter- and intra-cellular. $\times 350$. B, Section of sclerotium, from culture on potato agar, showing definite cortex and parenchymous medulla. $\times 300$.

The only spores produced appear to be microconidia. These have been observed only in culture. These microconidia were first found in a culture of the fungus on a synthetic medium,² in test tubes, and later on potato agar, especially when adjusted to a pH of about 5 by the addition of hydrochloric acid. The cultures in which microconidia have been found

² Water 1000 c.c., agar 15 grams, glucose 20 grams, peptone 10 grams, dipotassium phosphate 0.25 gram, magnesium sulfate 0.25 gram, as given by Cook and Taubenhause (1).

were from 25 to 40 days old. Small white granules, less than a millimeter in diameter (Plate XI, D), appear buried in the medium mostly at the top and back of the slant, adjacent to the wall of the tube. On examination under the microscope these granules are found to consist of numerous spores, borne on verticillately branching conidiophores (Fig. 2, A). The microconidia are approximately spherical, $1.7\text{--}3.7\ \mu$ in diameter, with an average of $2.5\ \mu$. Attempts to germinate them have failed, and, since they have not been found under natural conditions, the role they play, if any, in disseminating the fungus and in infection is unknown.

The mycelium is septate, and both inter- and intra-cellular. On media used by the writer it is white in color, with the aerial mycelium tipped with clay³ in older cultures.

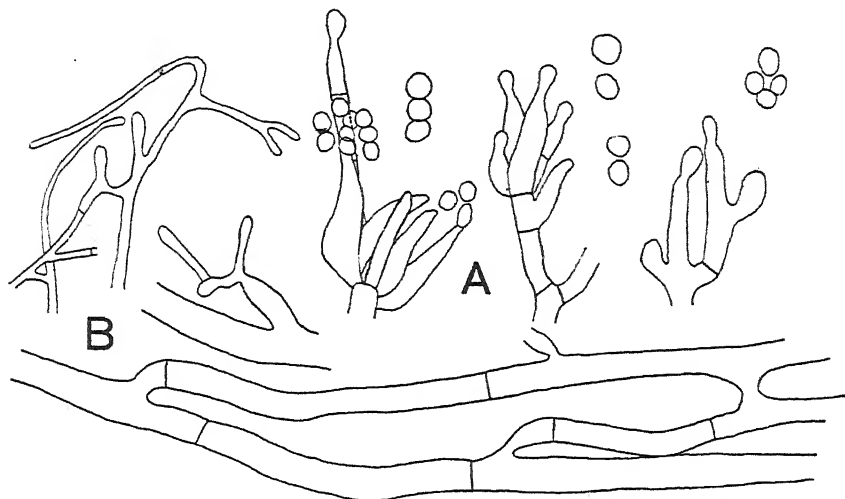


FIG. 2. A, Microconidia and portions of verticillately branching conidiophores. From a 40-day-old culture on potato agar, pH 5.04. $\times 1500$. B, Mycelium from a 7-day-old culture on potato agar to show manner of branching. $\times 500$.

Since no spore stage other than microconidia has been found, it seems necessary to continue the fungus in the form genus *Sclerotium* as was suggested by the writer in 1918 (4, p. 72). Because of the structure of the sclerotia the form genus *Sclerotium* is preferred to *Rhizoctonia*, even though the method of branching of the mycelium suggests to a certain extent that of the latter genus (Fig. 2, B). The species name *gladioli* is here proposed. The formation of sclerotia and microconidia, of the type figured (Fig. 2, A), strongly suggests that the fungus may be a species of *Sclerotinia*, but at-

³ Designations of color are according to Ridgway, Robert. Color standards and color nomenclature. 44 pp., 53 col. pl. Washington, D. C. 1912.

tempts to bring about further development of the sclerotia have been unsuccessful.

PATHOGENICITY

The ability of *Sclerotium gladioli* to infect the gladiolus and produce symptoms identical with those found under natural conditions has been established through numerous experiments both in the field and under greenhouse conditions. Wallace (9, p. 72), Fitzpatrick [according to Whetzel (10)] and Drayton (2, p. 205) report successful infection experiments. Infection takes place when corms are planted in infested soil, and when the progeny of healthy corms planted in clean soil are inoculated by placing mycelium in contact with them. Sound corms placed in sterilized moist sand in moist chambers and inoculated by pressing bits of medium carrying the fungus against the uninjured surface, when held at a temperature of 25° C., develop lesions in about ten days.

A phellogen layer is formed at the juncture of the diseased and healthy tissue, similar to that formed in hard rot (5, p. 168). Diseased tissue, especially in advanced cases, can be severed from the apparently healthy tissue beneath, the rupture occurring at this phellogen layer. Sections through lesions show from one to many layers of these thin-walled cells indicating progressive stages in the advancement of the fungus into the healthy tissues.

CULTURAL CHARACTERS

Sclerotium gladioli has been grown on a large number of media, both standard and special, the latter being used largely in an attempt to induce sporulation. The fungus is tolerant of a wide range of conditions, and growth is fairly uniform. The mycelium is mostly white, at first appressed and somewhat silky, later becoming more distinctly aerial (in petri dishes) in the outer one-third or so of the growth. The surface of the medium in a standard petri dish is covered in about 7 days, with appressed mycelium in the inner two-thirds of the diameter and aerial mycelium forming a ring covering the outer one-third or so of the surface. Later the area of appressed mycelium is increased somewhat, but the ring of aerial mycelium near and on the wall of the dish persists indefinitely. With age the color of the aerial mycelium is gradually changed from white to clay.³ Sclerotia appear in from 5 to 20 days, usually in the older parts of the culture following a darkening of the mycelium, although in some instances, *e.g.*, on cornmeal agar without sugar, they appear simultaneously throughout the culture and with no darkening of the hyphae (Plate XI, F).

The growth on oat-mush agar is more rank than on decoctions of potato and cornmeal, growth on the latter being scanty and relatively inconspicu-

ous. Sugars, 2 and 5 per cent, added to plant decoctions increase the growth rate slightly and hasten the formation of sclerotia (Plate XI, E, F). Fructose was found to be slightly better than dextrose and saccharose for sclerotial formation, and 2 per cent as effective as 5 per cent although the growth rate was somewhat increased with the larger amounts. With sugars added to decoctions of potato, cornmeal, and oats, a sclerotial crust is formed, while without sugars the sclerotia stand out as individuals. Zonation is sometimes present in cultures in petri dishes, but is not very conspicuous. Lactic acid, two drops to 15 c.c. of medium, slightly retards sclerotial formation, although the fungus is more tolerant toward a neutral or acid medium than toward one that has been made alkaline by the addition of sodium hydroxide. Saltations result frequently, especially where but a thin layer of medium in petri dishes is available to the fungus.

Cultures of *Sclerotium gladioli* have a very pronounced musty odor which may aid in the identification of the fungus. This odor is strongest in recently isolated cultures, becoming weaker as the growth ages. On several occasions recent isolations of the fungus have either failed completely or made irregular growths when cultures have been confined in closed containers of limited volume. Whether or not there is any relation between these failures and the presence of the substance responsible for the characteristic odor has not been established.

Reaction between cultures from different sources. Among other attempts to induce sporulation by *Sclerotium gladioli* two or more isolations of the fungus were grown at one time in the same petri dish. Although these attempts were failures so far as fruiting is concerned, it was found that certain isolations, when paired, resulted in the formation of a heavy dark line at the junction of the thalli, whereas other combinations did not react in this manner (Plate XI, A, B, C). It was thus possible to divide the cultures from numerous sources into groups, positive and negative in this respect, no two members of either group reacting with each other. The full significance of this characteristic, and whether or not anything other than the question of staling is involved, is not known.

Temperature relations. Cultures of *Sclerotium gladioli* were established by transferring bits of a solid medium carrying mycelium to the center of petri dishes of equal size containing equal amounts of hard potato agar (3 per cent) with 1 per cent dextrose. The medium was tested electrometrically and found to have a pH value of 7. After incubating all of the cultures for 24 hours at 20° C., they were divided into lots of four dishes each and each lot held for 10 hours at the particular temperature at which it was to be kept throughout the experiment. This permitted the fungus to establish itself in the medium in the petri dish and eliminated errors that might

arise from failure to allow sufficient time for temperature adjustments before making initial measurements. The diameters of the thalli were then recorded and the rate and amount of subsequent growth determined by measuring the increases over the original diameters. Four cultures were placed in each of a series of temperature chambers with the following temperatures: 0°, 5°, 10°, 15°, 20°, 25°, 27.5°, 30°, 32°, and 35° C. The diameters of the thalli at 72 hours were taken as a criterion for comparison, the results being represented graphically in figure 3. There is a gradual increase in rate of growth up to the optimum at 25° C., with a rapid drop beyond 27.5° C.

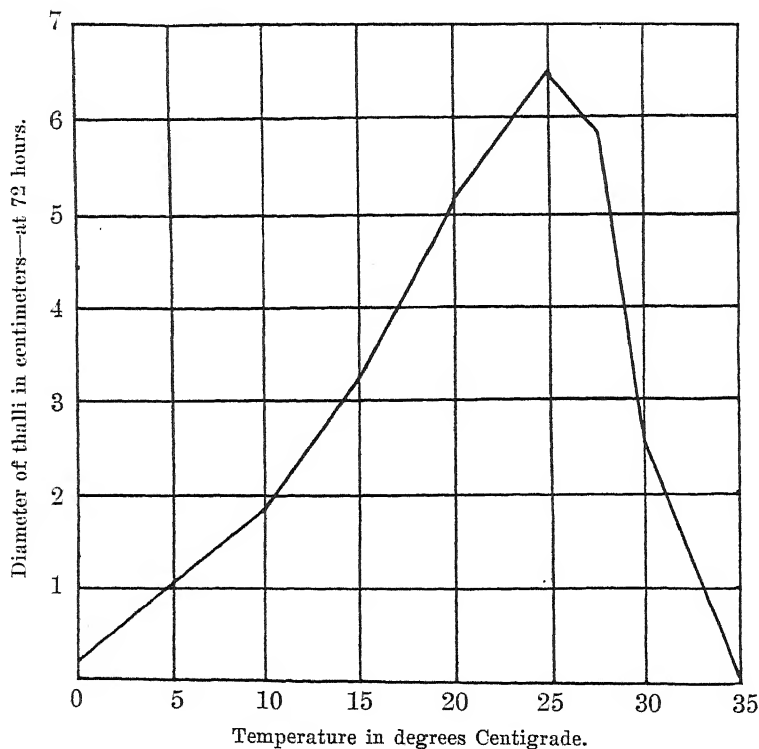


FIG. 3. Relation of temperature to growth of *Sclerotium gladioli* on hard potato agar with 1 per cent dextrose in petri dishes.

LIFE HISTORY

The dry rot fungus may be isolated from lesions on the corms at any time during winter or spring. This shows that the living organism is carried to the soil along with diseased corms at planting time. About 30 per cent of the offspring from diseased corms planted in clean soil are infected. The fungus does not grow directly from the old into the new corm. It either grows through the sheathing leaf bases or else out into the soil, from which

it attacks the newly developing corm. In connection with inoculation experiments it was observed that the fungus freely enters the soil, and experiments previously reported (5, p. 162, 180) show that the fungus will live in soil in which no gladioli have been grown for at least four years.

The fungus, then, passes the winter in diseased corms, in infested soil in the field, and doubtless also to some extent in infected stems. Just what rôle is played by the sclerotia, found especially on husks and the lower parts of the stems, is unknown. Further, since the microconidia have not been germinated, the part they play, if any, in the life history of the fungus remains unsolved. The fact that diseased corms almost always show numerous lesions suggests the possibility of infection by spores, but no evidence to pathogene, approximately 70 per cent of the progeny will escape infection. since sound corms planted in soil in which no gladioli have been grown have uniformly remained sound and produced disease-free offspring.

As previously reported (5, p. 173), hundreds of healthy corms have been housed over winter with many thousands of diseased corms without becoming infected. This indicates that *Sclerotium gladioli* is not disseminated in storage. It has been observed, however, that the percentage of infected corms is increased when considerable soil from the field is taken into storage on improperly harvested corms. The excess soil holds moisture, delaying drying of the husks, and under such conditions the fungous mycelium may actually grow from corm to corm. Lesions increase in size during storage especially where the temperature and humidity are high.

CONTROL

In connection with an investigation of the hard rot disease the writer included corms affected with dry rot in the several experiments on control measures (5, p. 172-180). The treatments of diseased corms with (1) formalin at the rate of 1 pint of commercial formalin to 15 gallons of water for 18 hours, (2) corrosive sublimate, 1-1000 solution, for 18 hours, or (3) chemicals, including sulfur, air-slaked lime, acid phosphate, and soot, in which the corms were rolled and with which they were covered after placing in the row and before covering with soil, were found to be of no value. These treatments were made in the spring before planting. Similar tests were made in the autumn immediately after digging, at which time the lesions are materially smaller than in the spring. Failure again resulted. Recently dug corms have also been treated with formaldehyde gas (5, p. 177), with water at 50° C. for one-half hour, and with dry heat at 50° C. for one and one-half hours. These treatments were unsuccessful in reducing the percentage of diseased corms, and the dry-rot fungus was isolated from numerous lesions, showing it to be viable still. Drayton (2, p. 208) reports

failure in the use of formalin, mercuric chloride, Uspulun, and Bayer compound, both for the treatment of diseased corms and as a drench for soil in which diseased plants had grown. No satisfactory material for the disinfection of soil has been found.

When corms affected with dry rot are planted in soil free from the pathogene, approximately 70 per cent of the progeny will escape infection. It is evident, then, that soil infestation is one of the most important factors to be considered in connection with control measures.

In an article on *Fusarium* rot of gladiolus corms (6) the writer has proposed general recommendations for control, involving sorting, crop rotation, proper harvesting and storage, which are applicable to the several corm rots, including dry rot.

SUMMARY

Dry rot is an important disease of gladiolus corms in the United States and Canada, and is known to occur in England, France, and Holland. A description of the disease is given. Corms and cormels become infected in the field, and the rot advances in storage. Plants in the field may die during the season either from a stem rot or the decay of the corms. Soil becomes infested from planting diseased corms, and the pathogene will survive in the soil for at least five years.

The pathogene causing dry rot is placed in the genus *Sclerotium* because of the structure of the sclerotium and the absence of spores other than microconidia. A new species, *gladioli*, is made. The morphological and cultural characters of the fungus are recorded.

On hard potato agar with 1 per cent dextrose, the fungus grows over a range of from about 0° to 35° C. Optimum growth takes place at about 25° C.

Infection was readily obtained on uninjured corms in the laboratory and on those growing in the greenhouse and experimental gardens.

No satisfactory treatment of diseased corms to disinfect them has been found. Crop rotation, avoiding the use of the same soil oftener than once in four or five years, is believed to be of major importance in holding dry rot in check. This practice should be combined with sorting to eliminate diseased corms, together with the proper handling of corms at harvest and during storage.

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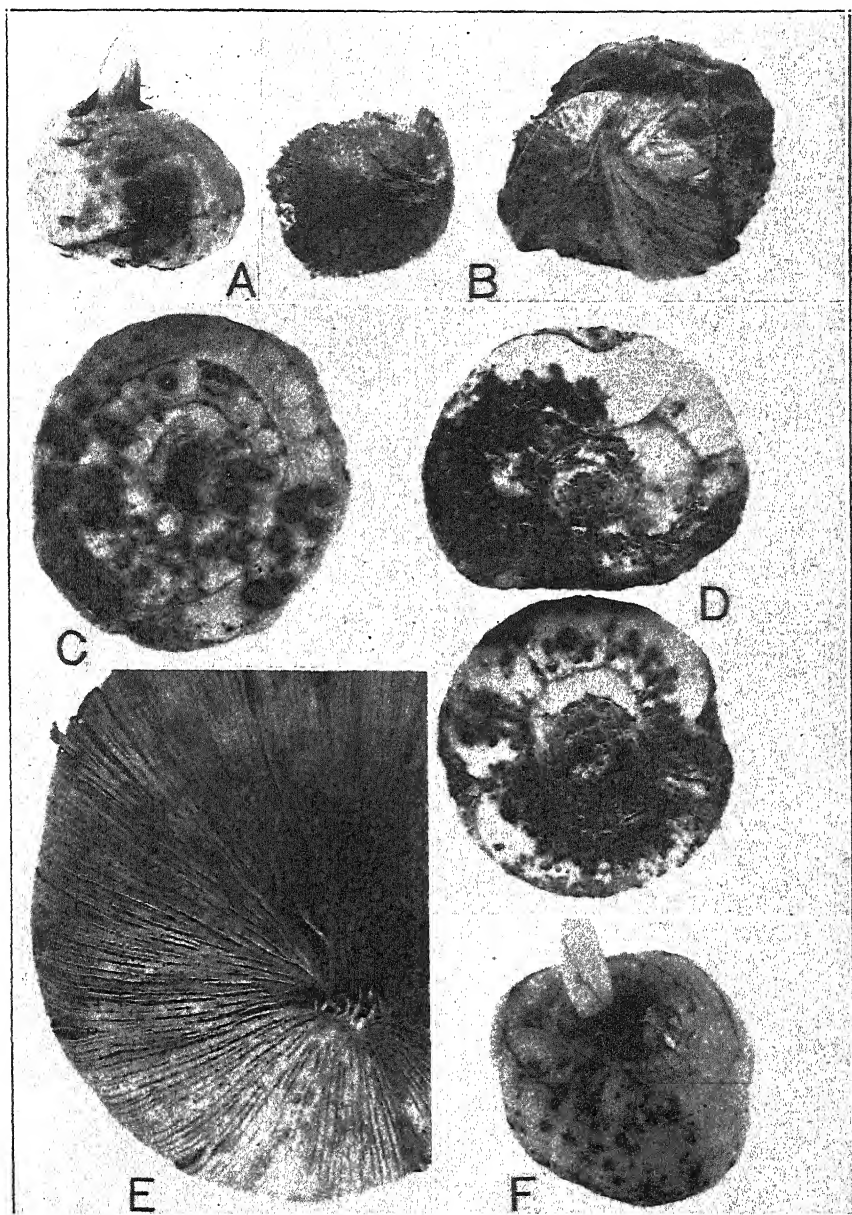
EXPLANATION OF PLATES

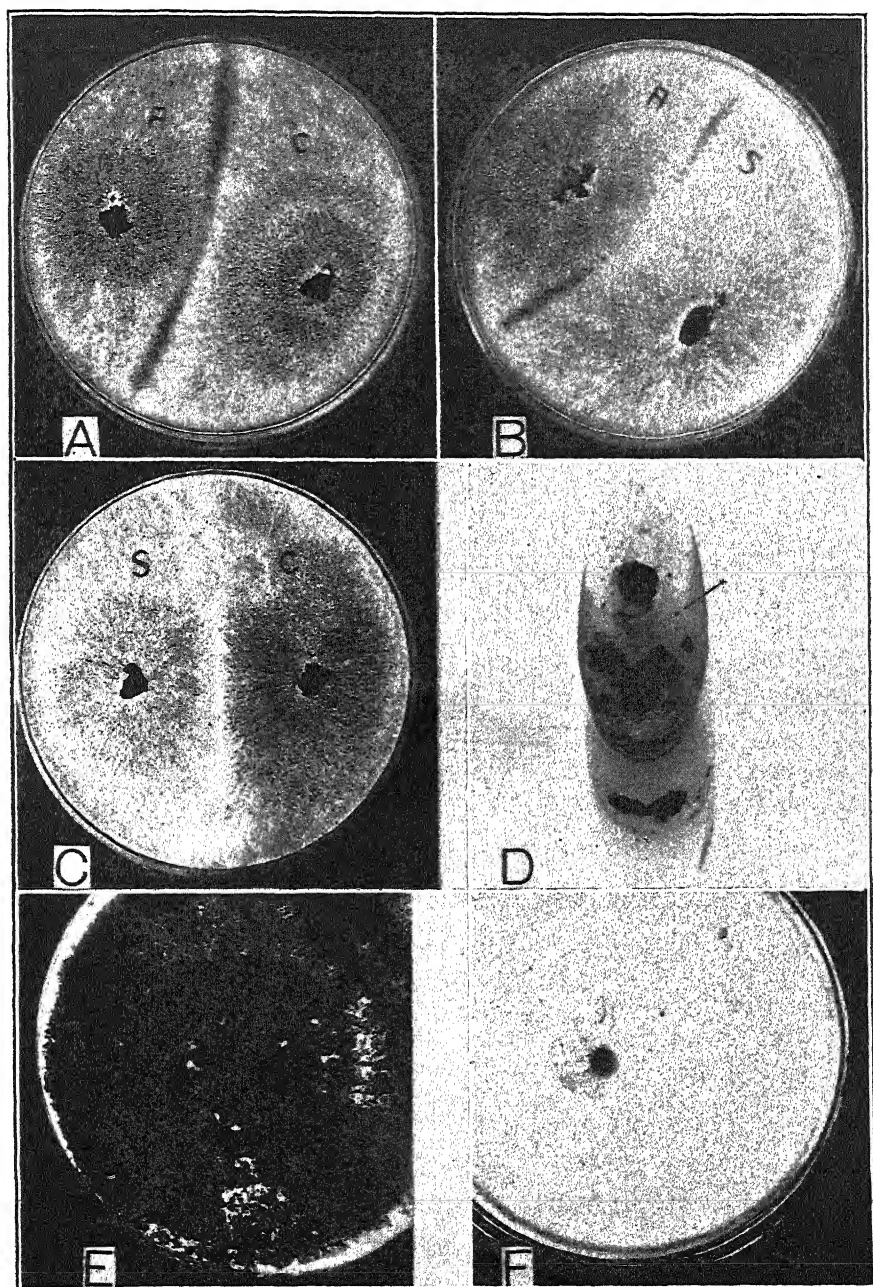
PLATE X

Corms affected with *Sclerotium gladioli*. Corms at lower left and upper right show sclerotia on husks. A, B, C, D, and F, approximately natural size; E, $\times 2$.

PLATE XI

Cultures of *Sclerotium gladioli*. A, B and C show results of growing paired isolations from different sources on potato agar in petri dishes. Note reactions, indicated by dark-colored junctures, between A and C, and A and S. Photographed after 10 days. D, Microconidia produced on potato agar, after 40 days. Aggregations of spores and conidiophores appear as small, globose, granules, white by reflected light, buried in the medium, as indicated by arrow. E, F, cornmeal agar with and without 2 per cent dextrose, respectively, after 40 days. Note effect on formation of sclerotia.





"BLACK TIP," A FINGER-TIP DISEASE OF THE CHINESE BANANA IN BERMUDA

LAWRENCE OGILVIE

INTRODUCTION

The "Chinese," "Canary," "dwarf," or "Cavendish" banana (*Musa cavendishii* Lamb), introduced into Bermuda probably about 1850, has furnished an important article of food since that time. It is grown, however, only for local consumption since the cost of production is excessive compared with similar costs in other banana-producing countries. At the present time there are about 50 acres of the more sheltered land in the valleys under cultivation in bananas, which produce some 35,000 bunches a year.

In the Annual Report of the Board and Department of Agriculture, Bermuda, for 1924 (7), reference is made to a somewhat serious disease, commonly known as "black tip," which was first brought to the notice of the writer in August of that year. There is evidence that the disease had been present in the Islands for a considerable number of years before that date but up to then had occasioned little loss.

Under Bermuda conditions the flowers of the female fingers of the Chinese banana either fall off, a break occurring at the base of the perianth segments and leaving a scar which rapidly dries up, or wither and remain attached to the fruits. The bunches are gathered when they are still green but when the fingers have become well filled.

SYMPTOMS

Under certain conditions a black discoloration becomes apparent just below the flower and progresses down the fruit. In about three weeks' time it may progress some two inches down from the tip. The diseased area is frequently irregular in outline, often affecting one side of the fruit more than another, and is bordered by a narrow grey or light yellow margin. An ill-defined zonation is frequently present. (Fig. 1.)

ASSOCIATED ORGANISMS

Investigations carried out in 1924 and at subsequent intervals when time was available show that a large number of organisms are commonly present on the withering flowers. These include species of *Fusarium* and *Cephalosporium*, *Aspergillus luchuensis*, Inui (identified by Dr. Charles

Thom), *Glocosporium musarum* Cooke and Masee, species of *Macrosporium* and *Alternaria*, and bacteria. The whole flower is frequently covered by the frass and silky webs of a Tineid larva. Predatory mites of the genus *Hypoaspis* (*Gamasidae*) are commonly found on the withering flowers and



FIG. 1. A bunch of the Chinese banana severely damaged by the "black tip" disease. The fruits were still green when the photograph was taken.

fruit tips. Other organisms which growers supposed might be implicated in the diseased condition are snails, chiefly *Polygyra appressa* Sag., very commonly found on the tips of the fruits, especially after rain, and the aphid *Pentalonia nigrinervosa* Coq., which clusters on the fruit-tips and is attended by ants. The exudation of latex, if the break occurs when the flower is still unwithered, also attracts large numbers of Drosophilid flies, chiefly *Drosophila ampelophaga* Loew. (See 8.)

It was soon evident, however, that none of the organisms other than the fungi were invariably present, except the mites, and these were found to be present on healthy and diseased fruits. With regard to the fungi and bacteria, inoculations of all those isolated in 1924 and again in 1925 produced no marked effect on green banana fruits.

A further outbreak of the disease in the summer of 1927 again drew the writer's attention to it; and further investigations showed that at a relatively late stage, when the discoloration had progressed about half-an-inch down the fruit, pale brown mycelial wefts were present on the surface of the fruit, extending to within about 3 mm. of the margin of the lesion, though at advanced stages they tended to be obscured by thick growths of *Cephalosporium*, *Fusarium*, and other fungi. Little difficulty was found in isolating this fungus, which proved to be identical with *Cercospora musarum* Ashby, first described by S. F. Ashby from Jamaica (3), where it was found to be associated with a "blackspot disease" of the leaves of the Gros Michel banana.

FRUIT INOCULATIONS

A large number of constantly successful inoculations with this fungus were carried out during August, 1927, both on detached fruits and on bunches in the field. Mycelium from pure cultures grown on 1 per cent dextrose potato agar produced a rapid discoloration when placed in small incisions in the skin of green bananas, the surfaces of which had been previously sterilized. Check inoculations remained normal. In less than two days after inoculation a black discoloration extended outwards from the point of inoculation, and in about three weeks a lesion some two inches in length was produced (Fig. 2). The successful results of these inoculations were referred to in a short note published in the Bermuda Agricultural Bulletin (6). The fungus has been reisolated constantly from these lesions. None of the inoculations with the other fungi was successful in producing the characteristic discoloration, although an *Alternaria* produced a slight surface rot.

SECONDARY ORGANISMS.

The whole fruit is seldom affected. The ripening of the fruit appears to put a stop to the progress of the lesion. At this stage, however, the skin

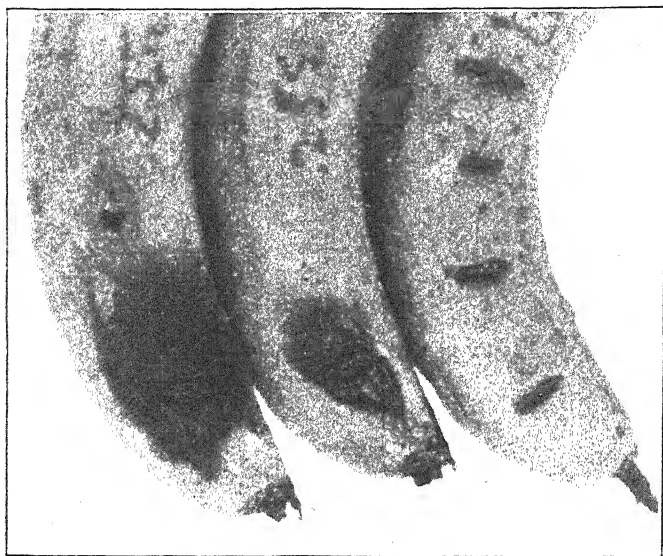


FIG. 2. Green fruits inoculated with *Cercospora musarum* Ashby. Check incisions at the left. Photograph taken a fortnight after inoculation.

of the affected area tends to split, and numerous insects are thereby attracted. The larvae and adults of *Carpophilus* beetles (*Carpophilus dimidiatus* Fabr., etc.), of *Araecerus fasciculatus* de Geer, and of *Drosophilid* flies are especially frequent. *Chaetopsis aenea* Wd. (Ortaliidae) has been bred out from such fruits. These and other insects, and also snails and rats, soon render the affected bunches quite unsalable.

THE FUNGUS

The fungus in question has been well described by Ashby in his account already referred to (3) and quoted by Nowell in his book on "Diseases of Crop Plants in the Lesser Antilles" (5). Nowell is at present carrying out a further investigation of the fungus with a view to determining its systematic relationships. It grows well on 1 per cent dextrose potato agar, producing a somewhat flocculent white mycelium which becomes dark olive green with age. In plate cultures the aerial mycelium produced from the centers of the colonies is a characteristic feature. Recently isolated cultures sporulate abundantly at summer temperatures (about 75° F.).

Microscopic examination of the affected areas of the fruit shows that the fungus is confined mainly to the green skin, where it brings about necrosis of the cells. Its hyaline to fuscous intercellular mycelium some 4 μ in

diameter may be readily seen in sections. The spores of the fungus are produced in abundance on the surface of the lesions during warm weather.

SYMPTOMS ON THE LEAVES

The fungus *Cercospora musarum* is associated with two distinct types of lesions on the leaves of the Chinese banana:

1. Minute round black spots scattered over the surface of the leaves and especially in the neighborhood of the midrib. These are probably identical with those referred to by Ashby as "pin-point round black spots on the main nerves of the leaf blade." The fungus has been isolated constantly from such spots, and conidia of the fungus are produced in abundance on the surface of such areas if pieces of the leaf are kept in a moist chamber.



FIG. 3. A typical leaf-lesion caused by *Cercospora musarum* Ashby.

2. Large brown lenticular lesions surrounded by a bright yellow margin (Fig. 3). Such areas usually commence at some point of injury, very frequently at the edge of a leaf blade or where it has split transversely, or from the mottled areas associated with a *Phyllosticta* (probably *Phyllosticta musae-sapientii* Frag. et Cif.). The fungus has been isolated constantly from the edges of such lesions. A surface mycelium identical with that found on the fruits is frequently found on such areas, as is also the characteristic zonation associated with the tiprot of the fruits. If pieces from such areas are kept in a damp chamber, an abundant crop of conidia may

be secured. It should be mentioned, however, that, during the winter months in Bermuda and under other conditions unfavorable to the growth of the plant, dead areas appear on the leaves which can be distinguished with difficulty from those caused by the *Cercospora*.

In his report of the "blackspot disease" in Jamaica, Ashby (3) described symptoms identical with the foregoing. He also described "round jet black somewhat convex spots with a central nipple" on fruits. These were found by him only on trees that had the black leaf spot. No such fruit spot occurs in Bermuda.

LEAF INOCULATIONS

Healthy leaves were inoculated with the fungus by cutting small nicks out of the leaf-margin and placing mycelium therein. One month from the time of inoculation (Sept. 7) distinct evidences of infection were observable on thirty such inoculated areas, while seven check wounds remained normal. Infection is first observable as black or brown streaks progressing inwards from the incised areas. Drying out of the infected areas soon takes place, the edges of the lesions being bordered by a bright yellow margin. The fungus was reisolated from the lesions without difficulty.

TEMPERATURE RELATIONSHIPS

The disease is most noticeable on both fruits and leaves during the summer months in Bermuda, late June, July, August, and September, when the mean maximum air temperature is above 75° F. By the beginning of October there is comparatively little discoloration of the tips of the very young fruits. The sales of the grower are most reduced in October and November when the greatest number of affected fruits ripen.

CONTROL MEASURES

In the light of the information obtained the following control measures have been recommended and have been followed with some success:

1. Fresh plantings should be spaced at least 8 feet by 10 feet apart in well-drained situations. As a general rule only two suckers should be left as "followers." It has been found that the disease causes most loss in old, closely planted and badly drained plantations.
2. No diseased fruits should be left lying about the fields.
3. As far as possible dead sheaths and leaves should be removed from the proximity of the bunches.
4. The large diseased spots on the leaves could be cut out with the aid of a large pruning shears, sickle, or similar tool.

Little or no control has been secured by spraying the young fruits with Bordeaux mixture to which a "sticker" was added.

SIMILAR DISEASES ELSEWHERE

A disease very similar to the black tip disease of Bermuda has been described from various localities. A *Stachyldium* is known to be associated with a tip-rot in the Canary Islands and the Gold Coast, but no successful inoculations have apparently been recorded. In the Canaries the female flowers are cut off the tips of the immature fruits since, "as they do not fall naturally, they would present an untidy appearance if left to wither attached to the fruit and would, moreover, form a harbour for insects" (1).

Mr. C. G. Hansford, formerly microbiologist in Jamaica, informs me that a tip-rot of the Chinese banana is quite common there, but has never been examined in detail as the variety is not of economic importance in that island. The occurrence of this disease in Jamaica is also referred to in an article entitled "The Banana and its Cultivation, with Special Reference to the British Empire" (2). It is said to be due to "a fungus which attacks the remains of the flower at the tip of the fruit and causes it to rot." Dr. John R. Johnston of the United Fruit Company mentions in a letter that he has noticed a similar trouble in Colombia.

Dastur (4) records the occurrence of a tip-rot of plantain in India associated with *Gloeosporium musarum*. He writes, "When the young flowers are infected, the infection is generally found to begin from the distal end, possibly arising through the flowers. The infected finger begins to turn black and shrivel from the distal end; as the infection progresses the whole finger turns black, shrivels and becomes covered with the pink spore-beds of the fungus. The attack rapidly spreads and involves the whole bunch." It was found impossible to produce lesions on green fruits of the Chinese banana by inoculations with pure cultures of *Gloeosporium musarum* under Bermuda conditions although the fungus is a common one on over-ripe fruits, thus confirming the work of R. A. Toro on this fungus (9).

It is probable that other fungi may be found to cause similar tip-rots elsewhere, but the widespread occurrence of *Cercospora musarum* on the leaves would lead us to suspect that the fruit rot would be found on the Chinese banana in localities where the fungus is present.

SUMMARY

1. A finger-tip disease of the Chinese banana (*Musa cavendishii* Lamb), commonly known as "black tip," is described from Bermuda. It is most prevalent during the summer months.

2. It has been found to be due to the fungus *Cercospora musarum* Ashby, which also causes characteristic leaf-spots.

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HISTOLOGY OF THE LESIONS PRODUCED BY SPHACELOMA FAWCETTII JENKINS ON LEAVES OF CITRUS¹

H. S. CUNNINGHAM

During the course of certain histologic investigations on diseased leaves, the attention of the writer was called to the condition existing in the leaves of *Citrus* affected with scab.

The lesions upon the leaves appear as hyperplastic areas somewhat lighter in color than that of the normal leaf, and have a corky appearance. The individual lesions are comparatively small but frequently coalesce to form a large corky patch. Where the lesions are numerous there may be considerable malformation of the affected leaf. The lesions are more commonly found on the under surface of the leaf although they do occur upon the upper surface as well.

The investigations reported in this paper were undertaken for the purpose of determining the histologic changes which take place in the infected leaf and which result in these characteristic warty excrescences. These studies include diseased leaves of three species of *Citrus*: rough lemon (*Citrus limonia* Osbeck), sour orange (*Citrus aurantium* L.), and Rangphur lime (*Citrus aurantifolia* Swingle). For purposes of comparison, wounded leaves of rough lemon were also studied.

Mature leaves bearing lesions were obtained through the courtesy of Dr. W. W. Yothers, of the Experimental Station at Orlando, Florida. This material was in excellent condition when received. Through the kindness of Dr. Anna E. Jenkins plants of rough lemon growing in the greenhouse at Cornell University were made available for wounding. The wounds were made on mature leaves by means of a common leather punch giving a circular hole two millimeters in diameter. In this way a clean cut wound was produced, care being taken to avoid cutting into or near the larger veins. The wounded leaves were removed from the tree after a period of eighteen days.

At the time of collection, or as soon thereafter as possible, small segments were cut from the leaves with a sharp razor. These segments included scab lesions or wounded tissue, as the case might be, together with

¹ This paper presents a part of the author's doctorate investigations on the pathological histology of leaf lesions. Grateful acknowledgement is made of the suggestions and criticisms of Professors H. H. Whetzel and A. J. Eames under whose direction the work has been done.

a portion of the healthy leaf on either side. Immediately after the segments were removed from the leaf they were placed in small shell vials containing medium chromo-acetic fixing solution (1). No vacuum pump was used to remove the air during the fixing process but the segments were kept submerged by placing a small wad of cheese cloth in the vial, thus forcing the segments below the surface of the liquid. Fixation was allowed to continue for thirty-six hours after which the material was thoroughly washed in running water and embedded in paraffin in the usual manner.

The studies were made from serial sections cut nine microns in thickness. Durand's method (2) was used in staining mycelium; crystal violet (6) for general histologic studies; and Sudan III as a test for suberin and cutin.

As the histologic changes are very similar in the diseased leaves of the three susceptibles studied, it appears sufficient to present in detail the condition found in leaves of rough lemon.

REVIEW OF LITERATURE

A search of the literature revealed no satisfactory account of the histologic changes in the leaf tissue which result from attacks of the scab fungus. Numerous writers make reference to the warty appearance of the lesion and the malformation of the leaf in severe cases of infection.

Swingle and Webber (8, p. 22) state that the leaf is often considerably thickened where the wart is situated and that, as the leaves persist for at least one year, cork formation proceeds farther than in the case of the fruit. Hume (5, p. 155) says "There is often a well marked conical depression on the opposite side of the leaf corresponding to the elevation on which the excrescence is situated." Fawcett (3, p. 46) mentions the warty appearance of the scab. Grossenbacher (4, p. 134) says "When the embryonic leaves of sour-orange or grape-fruit trees begin expanding the oil-glands often protrude above the outer surface of the emerging leaves. In some cases a few of these protruding oil-glands may break open, thus giving rise to crater-like conical elevations, the upper margins of which grow more or less and tend to close the pit. In cases in which the base of such a papillum grows very strongly the former oil-gland becomes elevated on a conical growth of superficial tissues and its tip becomes covered by flaky epidermal fragments arising in what was formerly the crater-like depression of the broken oil-gland." Stevens (7, p. 84) found that as the spots enlarged they became depressed on one side and raised on the other. Winston (9, p. 12) says "Distinct hyperplasia is often in evidence beneath the area attacked by the fungus, which fact probably accounts for the plainly evident excrescence associated with the scab lesions. Specialized host tissue

can frequently be found separating invaded from uninvaded parts." This condition he found to occur in older leaves but was not observed in leaves incompletely expanded.

THE HEALTHY LEAF

In order that the reader may better understand the histologic changes which take place in the region occupied by the lesion the normal anatomy of the leaf will be presented rather fully.

In normal structure the upper epidermal cells are very regular in size and shape. They are only slightly longer than broad and are more or less rectangular in outline. The outer wall is very thick with a heavy cuticle while the radial and inner walls are also somewhat thickened. In the majority of these cells the protoplasm forms a thin peripheral layer but in others the contents are dense and granular.

The palisade parenchyma occupies approximately one half of the total thickness of the leaf, the number of cell layers varying from two to three. The cells in the two upper layers are closely packed and are considerably longer than broad while the cells of the lower layer, where such a layer exists, are shorter and broader. The cell walls are thin and the chloroplasts, while not numerous, are rather large. At intervals in the upper layer of cells and just beneath the epidermis, small cavities occur. These cavities occupy the space of two or three palisade cells and are evidently lysigenous in character.

The spongy parenchyma is comparatively open in structure and is made up of thin walled cells which are more or less oval in shape and fairly regular in outline. Each of the cells contains a large central vacuole with a layer of cytoplasm next the wall in which the chloroplasts are embedded. The cells which lie nearest the palisade layer are much larger than those lying near the lower epidermis.

The lower epidermis is similar in structure to the upper with the exception that the cells are not so thick and that some of them are more elongated. Their outer and radial walls are somewhat thickened and a relatively thin cuticle is present.

Throughout the leaf large oil-cavities are numerous. These may lie wholly within the palisade layer, wholly within the spongy parenchyma, or may occupy a portion of both of these tissues.

In the region of the larger veins only the outer layer of palisade cells is evident and these are very much shortened. The bundle proper, together with the supporting cells of thick walled parenchyma and fiber cells make up the remaining thickness of the leaf at this point.

THE DISEASED LEAF

A cross section of an infected leaf made through one of the lesions on the under surface shows a marked change from the normal at this point. This portion of the leaf is much thickened, bulging out on the lower surface with a corresponding depression on the upper surface. There has been a marked increase in the number of cells and also a marked change in the character of the cells of the tissues involved (Fig. 1).

The upper epidermal cells are apparently unchanged except that anticlinal division has taken place. The walls of the palisade cells are thickened and the chloroplasts are either reduced in number and size or have disappeared entirely. Anticlinal division has occurred and in occasional cells periclinal division as well.

The most noticeable change has taken place in the spongy parenchyma. Infection has resulted in a large increase in the number of cells as well as an increase in their size. This increase in number of cells appears to be due to division in several planes and as a result the leaf is very much thickened at this point and intercellular spaces are almost entirely wanting. In addition to the increase in number and size of cells the walls have been very greatly thickened. This thickening is entirely cellulose in nature without any indication of lignification. Many of the larger cells have been further divided by cross walls which are somewhat thinner than the main wall but of the same cellulose nature. These thick walled cells are living and each contains a peripheral layer of cytoplasm in which the nucleus is plainly visible. Early in the development of the lesion a phellogen is formed in the spongy parenchyma and as a result of the activity of this phellogen a definite phellem is laid down, thus completely isolating the portion invaded by the pathogene. The cells lying without this phellem are non-living and have suberized walls. The hyphae of the causal organism are abundant in the outer portion of this mass of suberized cells but no trace of them could be detected in the tissues lying within the phellem.

The histologic changes which take place when infection occurs on the upper surface of the leaf do not differ essentially from those already described. In such cases the phellogen is also formed in the spongy parenchyma and extends upward on either side to the upper epidermis. The phellogen thus lines a cup-like cavity filled with a mass of dead cells intermingled with hyphae. There is distinct bulging of the leaf on the under surface due to the hyperplastic condition in the spongy parenchyma.

In certain cases the phellem may extend entirely through the hyperplastic portion, thus forming a layer from the upper to the lower surface and extending as a continuous band about the edge of the lesion. Such a condition would account for the shot-hole effect sometimes accompanying this disease.

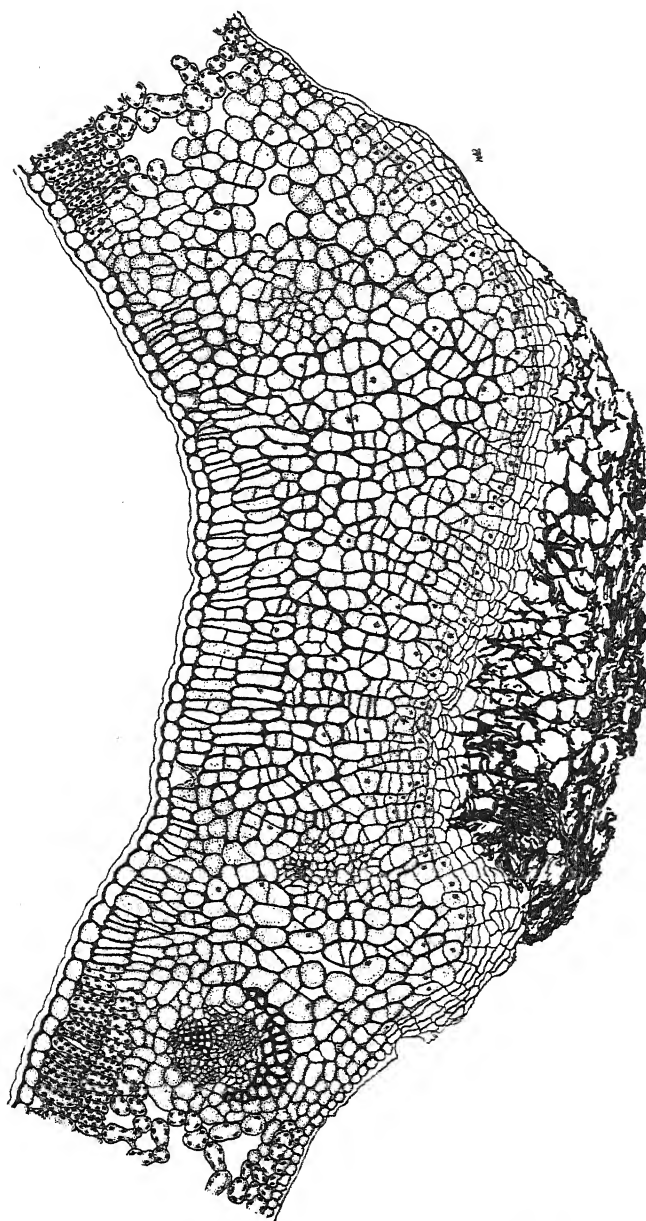


FIG. 1. A cross section through the scab lesion on a leaf of *Citrus limonia* showing the histologic changes induced by the pathogene. $\times 170$.

THE WOUNDED LEAF

The reaction of the leaf to artificial wounding is very different from that which ordinarily takes place when infection by the scab pathogene occurs. Hyperplasia occurs, but of a very different type. A cicatrice, or band of modified cells, is formed about the wound and occupies the full depth of the leaf from one epidermis to the other (Fig. 2). This cicatrice

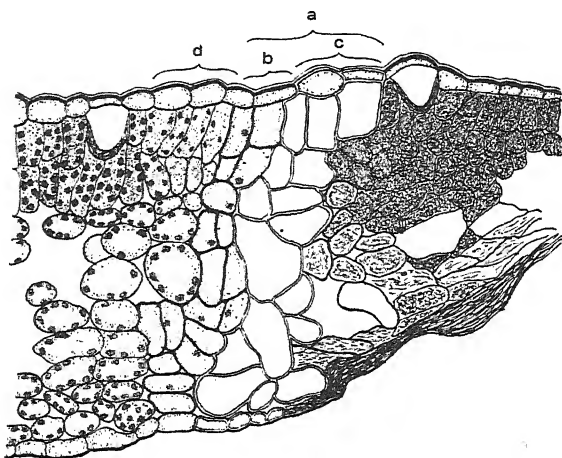


FIG. 2. Cross section through a leaf of *Citrus limonia* showing the wound periderm formed as a result of artificial wounding. a, wound periderm layer consisting of (b) phellogen and (c) phellem layers; d, modified mesophyll cells. $\times 300$.

is formed at some little distance from the edge of the wound. The intervening cells are dead and contain a dense granular substance which resembles tannin. All of these cells, particularly those of the spongy parenchyma, are more or less collapsed, a condition which is more complete towards the edge of the wound than nearer the cicatrice.

The cicatrice may be divided into two parts: the outer part (Fig. 2, a) lying nearest the wound and consisting of a typical wound periderm with its phellogen (Fig. 2, b) and phellem (Fig. 2, c) layers; and the inner part (Fig. 2, d) lying between the wound periderm and normal tissue and in which the cells, although modified, do not show clear evidence of having originated from the phellogen.

The wound periderm consists of a layer of phellem made up of a number of large cells devoid of all contents and with heavily suberized walls. The walls of the epidermal cells adjacent to this layer are also suberized. The remainder of the wound periderm is composed of somewhat irregular shaped, thick-walled, living cells. This layer of thick-walled cells may

vary from one to several cells in width but is usually widest in the region of the spongy parenchyma. No chloroplasts are present in this layer.

In the inner layer of the cicatrice the cells vary in size, are thin-walled, and the number of chloroplasts is much reduced.

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ROOT AND CROWN INJURY TO APPLE TREES

H. E. THOMAS

In an earlier paper (4) on root and crown injury of apple trees it was concluded that: (1) the killing of roots and crowns of apple trees in New York State is initiated in most cases by low temperatures; (2) certain fungi such as *Hypholoma sublateritium* Fries may aggravate the injury; (3) standard orchard practices and approach grafting of injured trees aid in preventing injury and in restoring trees already injured. Some additional observations and results of experiments are presented here.

RELATION OF VARIETIES OF APPLES TO INJURY

Since all varieties of apples are presumably grown on the same heterogeneous sort of stock it is to be expected that the roots of all varieties will suffer about equal injury by low temperatures. This seems to be borne out by field observations; and yet trees of such tender varieties as Ben Davis, Tompkins King, and Twenty Ounce are more likely to die outright than are the trees of the relatively hardy varieties MacIntosh, Northern Spy, Greening, and even Baldwin. Two reasons for this are apparent. First, in the case of the tender variety, the direct injury is not restricted to the stock but in extreme cases may extend up the trunk to the crotch. Second, there is a tendency on the part of certain varieties to produce cion roots which are, in many cases, distinctly more hardy than the roots from the stock itself (2). These cion roots are found growing vigorously from MacIntosh trees, for example, on which the crab roots below are entirely dead. The possibility of an effect of the cion on root characters (3) deserves further study in this connection.

RELATION OF FUNGI TO INJURY

Although *H. sublateritium* is abundant in some localities on dead and dying wood of various trees, we have identified it on apple trees from only three sources. Two of these cases were in orchards in which only a single tree was involved. In the third orchard a block (Block 1) of 136 Baldwin trees about 50 years old has been under observation since May, 1925. At that time four trees had died in a localized area which appeared to be similar in soil type and drainage to other parts of the block. In September, 1927, two additional trees adjoining this area had died. *H. sublateritium*

was present on all of these six trees before they died and undoubtedly hastened their death. However, other trees in the same block showed severe injury at the crown and no evidence of the fungus; while, conversely, other trees bore an abundance of the mycelium in the outer cortex of the roots but showed little, if any, deeper injury and no apparent effect on the top. In inoculation experiments with this fungus (July, 1925) it has been found (November, 1927) to penetrate slowly through the outer cortex of large roots on old trees, but deeper penetration seems dependent on rather severe injury to the cambium and wood of the roots. In addition to the inoculations already described (4), on which the preceding notes were made, a single vigorous tree in Block 1 was inoculated by burying a rotting root in contact with a small wound on a large lateral root. At the end of the second growing season the mycelium could be found at the margin of the wound, but had not penetrated more than 5 millimeters in any direction.

This block of trees was mapped on June 4, 1926, and again on September 28, 1927, to show the number of trees injured at the crown and the number of injured trees which bore signs of the fungus. Of 46 injured trees of the first record, the fungus was found on 16. At the time of the last record the fungus was found on 12 out of 47 trees.

An attempt was made to inhibit the fruiting of *Hypholoma* by the application of powdered copper carbonate (100 and 200 gm.) or mercuric chloride solution (12 liters, 1-1000) around the bases of infected trees. After two seasons there was some fruiting following each of the treatments.

Occasionally other fungi of the mushroom type are found in a relation which resembles that of the *Hypholoma* as described above. However, these have not been found on more than an occasional tree in any orchard.

RELATION OF CULTURAL PRACTICES TO INJURY

The killing of roots and crowns of apple trees is profoundly influenced by the conditions which favor or retard the growth of the trees and the season of the year during which these conditions prevail. Thus a vigorous growth is of greatest importance in preventing injury; while an excessive growth or a late growth, which tend to prevent adequate ripening of the tissue in autumn and early winter, may prove dangerous.

The effect of cultivation on the degree of injury is well illustrated by two adjacent blocks of Baldwin (Block 2) and Wealthy (Block 3) which have been under observation since 1922. At that time Block 2 was in good cultural condition, and it has been cultivated every year except two (1925 and 1927) since that time. Block 3 had fallen into neglect before 1922 and has been cultivated only one season since that time. The soil of the two blocks appears to be similar in type and slope, and the trees are of about the

same age (15–20 years). Wealthy is considered by Chandler (1) to be very hardy, while Baldwin is only moderately so.

TABLE 1.—*Relation of tillage to root and crown injury of apple*

Block	Variety	Tillage	Number of trees				
			Total	July, 1922		September, 1927	
				Dead	Injured	Dead	Injured
2	Baldwin	Fair to good	208	1	2	5	8
3	Wealthy	Very poor	198	6	15	15	88

Table 1 shows the relative amounts of injury in the two blocks in 1922 and 1927. The variety Duchess had been grown as fillers in Block 3 up to 1926. In 1922 and again in 1925 this variety showed approximately three times as much injury as was found in the Wealthys.

While the emphasis here is placed on cultivation, observations indicate that applications of quickly available nitrogenous fertilizers have a similar effect in preventing injury or promoting recovery.

Faulty drainage in orchards has long been recognized as an important factor in bringing about the death of roots. However, only extreme cases have been given particular attention. It is becoming more apparent that a high water table or water-logged soil even for a short period of time may permanently damage the lower roots, thus weakening the tree and forcing the root system into a more exposed position (1). Orchards in which poor drainage appears to play an important part in bringing about root and crown injury are common in the principal fruit belt of western New York.

PREVENTION AND RECOVERY

Assuming that the orchard site has been selected, three things deserve special emphasis in preventing injury and in promoting the recovery of injured trees. The first two, adequate drainage and the maintenance of optimum growth conditions, have already been touched upon. The third, approach grafting of injured trees, has received more particular attention since 1923 and will be treated more in detail. Two types of grafts were used—ordinary apple seedlings such as are used in the nursery, and cion-rooted trees of the varieties MacIntosh and Delicious. Most of the grafts were made in 1924 and 1925. In September, 1927, 216 seedlings and 122 MacIntosh and Delicious grafts were examined in 6 orchards in western New York. Of these, 78 per cent of the seedlings and 81 per cent of the MacIntosh and Delicious grafts had made a good union. There was no significant difference between the MacIntosh and Delicious grafts. Growth

of all grafts varied widely with individuals, with soil conditions, and particularly with the condition of the tree on which the grafts were made. The treated trees ranged from slight injury to complete girdling at the crown. Trees with severe injury made fewer unions with the grafts, and the grafts which persisted made distinctly less growth on these trees. The maximum diameter attained by all grafts of 1924 and 1925 was 2 inches, with the average near 1 inch. The response of many treated trees is slower than was at first expected. In three orchards in which the trees are 40 to 60 years old it is yet too early to show how much benefit may be expected from this treatment. The grafts grow about as well on these old trees as on younger trees, but for obvious reasons the response of the trees is much slower. In one (Twenty Ounce) of the three younger orchards the conditions have been such as to produce additional injury on many trees. Most trees with severe injury have continued to decline in spite of treatment, and

TABLE 2.—*Terminal growth (in inches), over a five-year period, of an injured tree treated by approach grafting in 1923*

	Terminal number	1923	1924	1925	1926	1927
Least injured side	1	30.0	17.0	5.5	5.5	9.5
	2	17.0	14.0	5.5	5.5	11.5
	3	10.0	5.5	2.0	15.0	24.5
	4	10.0	1.0	5.5	6.0	19.0
	5	24.5	7.5	1.5	4.0	16.0
	6	3.5	1.0	4.5	7.5	8.5
	7	5.0	4.5	4.5	5.5	9.0
	Averages	14.2	7.2	4.1	7.0	14.0
Most injured side	8	3.0	0.7	1.0	0.7	0.7
	9	0.7	0.7	2.5	5.0	13.0
	10	0.7	0.5	0.5	2.0	9.5
	11	0.7	2.0	6.5	5.0	17.5
	12	3.0	1.0	7.5	3.5	17.0
	13	1.0	1.0	6.0	6.5	14.5
	14	1.5	6.0	8.0	9.5	12.0
	15	0.7	7.0	7.5	9.0	14.0
	16	1.0	1.0	5.5	6.5	20.0
	17	0.7	1.0	5.5	3.5	11.5
	18	1.5	1.0	8.0	6.5	16.0
	19	1.5	1.0	9.0	6.5	15.0
	20	1.0	2.5	2.5	9.0	9.0
	21	8.0	1.0	6.5	5.0	9.0
	Averages	1.7	1.8	5.4	5.5	12.7

it now seems that approach grafting in this orchard is of doubtful value until some of the adverse conditions, such as poor drainage, are altered. Even here, however, 80 per cent of 105 grafts have survived, and made a growth approaching that in other orchards.

In the two remaining orchards, mainly Wealthy and Greening, 2 of the 25 treated trees which were examined had blown over as a result of almost complete killing of the root system. The others with one exception were making a satisfactory recovery. An example of the best results which have been obtained from approach grafting is shown in table 2.

The measurements of annual growth of terminals were made on a Greening tree 8 to 10 years old on which at least three-fourths of the crown had been girdled and only one larger lateral root remained alive. Six seedlings were grafted into this tree in May, 1923. In September, 1927, four of these grafts were found measuring respectively 4, 3, 2.5, and 0.6 inches in diameter. It will be noted that little growth occurred on the weaker side of the tree during the first two seasons, and it may be argued that severely injured trees will not persist long enough to permit the grafts to become effective. On the contrary, many severely injured trees have been observed to survive without treatment for at least four or five years. Old trees are frequently seen with large dead areas at the crown dating back at least as far as the severe winter of 1917-1918.

SUMMARY

Additional evidence is offered to show that fungi are, at most, secondary agents in the production of root and crown injury in New York State.

The maintenance of optimum growth conditions including adequate drainage is emphasized for the prevention of injury and for promoting the recovery of injured trees.

For restoring injured trees the foregoing may be profitably supplemented by approach grafting. It is believed, however, that, except under the best orchard conditions, trees more than half girdled are doubtful subjects for treatment.

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THE MIGRATION OF BACTERIUM TUMEFACIENS IN THE TISSUE OF TOMATO PLANTS¹

J. BEN HILL

LITERATURE REVIEW

The literature on the crown gall of plants is extensive and in most of its phases is well known. References to the migration of the causal organism, *Bacterium tumefaciens*, Sm. and Town., (*Pseudomonas tumefaciens*, Sm. and Town., *Phytomonas tumefaciens*, S. A. B.) through the tissues of the host are to be found mainly in the reports of investigations by Smith, by Riker, and by Robinson and Walkden.

Smith and his associates (11), in the extensive earlier literature on *B. tumefaciens*, regarded the organism as an intracellular parasite. Their conception of the migration was that the infected tissue proliferated in a manner similar to that of cancerous animal tissue, pushing its way into and through healthy tissue, carrying the bacteria along within the cells. They state, "It is probable that the parasite in its migration from one part of the plant to another does not make free use either of the vessels or of the intercellular spaces, at least, we have not been able to find it in them. Rather, we think it is imprisoned within the specially stimulated and rapidly dividing cells and is by the growth of these cells carried along." Again, Smith (8) states, referring to the crown gall organism, "From what I have seen, I believe it occurs only inside the parenchyma cells, stimulating them to divide and passing on from mother cell to daughter cell in this manner." Smith (9) points out many similarities of the crown gall disease to the cancers of animals, especially emphasizing the connections between the "primary" and "secondary tumors." These connections were termed "tumor strands" and were regarded as proliferating tissue of the "primary tumor." Referring to this situation and regarding the

¹ Contribution from the Department of Botany, the Pennsylvania State College, No. 61. Published by permission of the Director of the Agricultural Experiment Station as Technical Paper 448.

migration of the organism, he states, "The organism causing this tumor occurs only inside of certain of the proliferating cells. When the cell divides, the organism is carried over into the daughter cells, in at least a part of which it multiplies."

Cook (1), in a recent report of an investigation of the early stages of crown gall, accepts the conception of Smith regarding the occurrence of "tumor strands" and "cancer tissue." Levine (2), investigating crown gall with special reference to its resemblance to animal cancers, does not accept Smith's view on "tumor strands" and "secondary tumors" in the crown gall disease. He says, "In no case have I found a growth of crown gall tissue attributable to an inoculation at some distant point." Levine makes no statements as to the methods of migration of the organism. Riker (4, 5, 6) and Robinson and Walkden (7) have shown that the bacteria are consistently located in the intercellular spaces and not within the cells of the host as Smith supposed.

Recently two distinct conceptions of the actual manner in which the bacteria migrate through the host tissues have been added to the earlier hypothesis proposed by Smith. Riker (4, 5, 6), as one of the results of extensive morphological and cytological investigations of crown gall on tomato which emphasize the relation of the causal organism to its host tissue, has reported his observations on the occurrence of *B. tumefaciens* in plant tissue and gives something of his conception of the manner of its migration. He observed the presence of a water-soaked area surrounding the puncture after inoculation. His conclusion was "that this darkened area, which tended to be parallel to the long axis of the plant, was caused by the occupancy of the intercellular spaces by liquid." He stated, upon further investigation, that the resulting tumor or gall at first corresponded in size and shape to this water soaked area and "closely coincided with it in outline." He conducted experiments the purpose of which was to increase the area having water in the intercellular spaces. Bruising and freezing the portions of the stem followed by inoculation with *B. tumefaciens* resulted in the formation of galls over an extended area. Riker implies that the migration of the bacteria is through the fluid which has been released into the intercellular spaces by the puncture of the needle. He says, "These experiments indicated that the bacteria could travel several centimeters at least if they were given a continuous channel of fluid through which they might pass." Again referring to the presence of the bacteria in relation to gall formation, he says (4), "The observed facts seemed rather to indicate the possibility that the organism began its activities in the liquid which occupied the intercellular spaces around the puncture." Riker (5), after referring to the inoculation of a stem by

puncture, states, "It seems unquestionable that the bacteria once inside such a wound, would migrate to the limits of the avenue provided by the flooded intercellular spaces." Riker again (4) states, "In order to understand more clearly the relations of the organism to the liquid in the intercellular spaces, it seemed advisable to make observations on its motility both in water and in expressed plant juice." Therefore, measuring the rate of motion of *B. tumefaciens*, he found (4, page 123) that motile bacteria in sterile distilled water or expressed tomato sap move approximately at the rate of 1 mm. per minute. After giving the results of these measurements, he states, "This gives an easy explanation of how the organisms might reach the limits of the region flooded with the liquid which was released by the puncture." Nowhere does Riker report any direct observation of the manner or method of the migration of *B. tumefaciens*, but from his statements one is led to infer that he conceives of the migration of the bacteria as due to the motility of the individual organism in (or through) the water or cell sap released into the intercellular spaces by the puncture of the inoculating needle.

Riker (4) also conducted some experiments designed to determine whether the bacteria had to be inserted into a wound or if they could enter a wound from the external surface of the stem. He demonstrated in 50 instances, properly checked, that they enter a wound from the outside of the stem and produce a gall in 100 per cent of the trials. He says, "The forces which govern this entry of the organism into the tissue are not definitely understood. The bacteria might conceivably be influenced by any or all of such factors as the collapse of drying tissue, negative pressure, sap rise, and motility with or without a chemotactic stimulus." In a second set of experiments he obtained galls in 50 per cent of the cases in which numbers of *B. tumefaciens* were applied to the surface of the stem at the needle punctures six days after they had been made. That cell sap released into intercellular spaces by a needle puncture might be resorbed in a period of six days, is apparently not considered a possibility.

Riker (4) also reports the passage of *B. tumefaciens* through the vessels of the xylem in the tomato stem.

In a series of experiments designed to determine the effects of inoculating water-soaked plant tissues with *B. tumefaciens*, Levine (2) found that the gall was produced only at the point of entry of the inoculum, and not throughout the entire water-soaked area as reported by Riker. Levine concludes that the size of the gall is related to the age of the tissue, larger galls developing in younger parts of the stem. Levine's results on this point cast doubt on the correctness of Riker's conclusion as to the importance of the water-soaked area in the migration of the bacteria.

Robinson and Walkden (7), in a critical report of their investigations of crown gall, express an entirely different conception of the migration of the causal organism. They studied the disease in *Nicotiana affinis* and in *Chrysanthemum frutescens*. Their reference to the migration of the bacteria is rather incidental to the main topic of their report, which deals primarily with the location and distribution of the bacteria in the tissues and their relationship to the "secondary galls" and the so-called "tumor strands." Their conception of the migration is that the bacteria advance through the intercellular spaces as zoogloae. They state, "Pl. VI, Fig. 22, shows, more highly magnified, the zoogloal strand of *B. tumefaciens* seen advancing through a large, longitudinal, intercellular space in the pith of *Nicotiana*." Again they state, "The bacteria often associated as zoogloea-like strands have, . . . been traced for shorter or longer distances from the point of inoculation. The course of this bacterial extension is by way of intercellular spaces and protoxylem." In their summary these investigators state, "Both in *Chrysanthemum frutescens* and in *Nicotiana affinis* we have definitely demonstrated, by staining, zoogloal strands of *B. tumefaciens* intruding along intercellular spaces and protoxylem vessels, forming centers for pathological disturbances and gall production along the tract."

These recent investigators, Riker (4, 5, 6), Robinson and Walkden (7), and Levine (2) all disagree with Smith (8, 9, 10, 11) as to the nature of the "tumor strands" and "secondary tumors" in the crown gall disease and consequently as to the manner of migration of the bacteria. Riker concluded that the tissue of the tumor strand arises as a result of a stimulation to division of the cells along the path of migration of the organism and not as proliferating strands of tumor tissue as Smith stated. Robinson and Walkden found secondary tumors, but regarded them as due to the migration of the bacteria through the intercellular spaces, especially through the pith and not as due to a proliferating tumor strand.

Robinson and Walkden agree with Riker as to the region but not as to the exact manner of migration. In contrast to the earlier conception of Smith that *B. tumefaciens* is an intracellular parasite, Robinson and Walkden as well as Riker have agreed that the migration is through the intercellular spaces and the xylem vessels. Riker, however, implies that the migration is due to the motility of the individual organism through water or released cell sap in the intercellular spaces, while Robinson and Walkden regard the migration as an intrusion of organized masses of the bacteria or zoogloae into the intercellular spaces and even the vessels.

Smith (10), referring to the presence of the bacteria within the cells, in one of his last contributions adds a footnote as follows: "Recently Riker

in the United States and Robinson and Walkden in England have denied this, maintaining that the organism is always between the cells and the subject is still in dispute."

INVESTIGATION

*Materials and Methods*²

In this investigation I studied particularly the method of migration and the rate of migration of *B. tumefaciens* through the host tissues and I am confining this paper to these points. The investigation was started with material from plants grown during the spring of 1926 and the observations checked from material obtained during the spring of 1927. For the host plant I used the tomato *Lycopersicum esculentum*. The bacteria³ used were grown from two to four days on nutrient agar slants in tubes and inoculated into the internodes of the young stems of the tomato. The tomato plants were grown in the greenhouse and hot bed under optimum conditions and were in all cases vigorous, healthy young plants. Numerous plants inoculated with these cultures as checks developed the typical tumors of the crown gall disease. Inoculations were made near the tips of the stems in internodes about 1 cm. in length. In making the inoculations, no attempt was made to limit the dosage of bacteria. In fact, maximum dosage was usual. Later work has convinced me that the first internode above the cotyledons of young seedlings about 3 inches in height furnishes the most uniform material. The material for microscopic study was taken at intervals of 15, 30, and 45 minutes, 1, 1½, 2, 3, 4, 5, 6, 9, 12, 18, 24, and 48 hours after inoculating. Pieces of the stem about 1 cm. in length, each containing the inoculation puncture, were killed in Flemmings' solution and imbedded in paraffin. Especial care was taken to secure abundant material of the stages shortly after inoculation, especially within the first hour. Microtome sections of this material 7 to 15 microns thick were

² Acknowledgements are due Miss Frances P. Gibbons, Mrs. Helen D. Hill, and Miss Ruth I. Foelt for assistance in parts of the microtechnical work. I am also indebted to my colleagues in the Department of Botany, The Pennsylvania State College and to Dr. C. R. Orton of the Bayer Chemical Company, New York, for their criticism of this manuscript.

³ The culture of *B. tumefaciens* used in this investigation was one isolated from *Geranium soleroi* and furnished to me by Dr. H. W. Thurston, Jr. Cultures of the causal organism used in the checks were sent to me by Miss Nellie A. Brown, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C. Throughout this paper I have used the name, *Bacterium tumefaciens*, according to the nomenclature proposed by Dr. Erwin F. Smith. I recognize that the older name *Pseudomonas tumefaciens* is extensively used and also that use of the newer name, *Phytomonas tumefaciens*, may in time supersede the others.

stained in Flemmings' triple stain. This staining method is an adaptation of Nixon's (3) technique for staining bacteria *in situ*.

During 1927, I checked the entire investigation from material secured during the spring of that year. In the checks I used the castor bean, *Ricinus communis*, and the flowering tobacco, *Nicotiana affinis* (Sanderii hybrids), as additional hosts and a different strain of bacteria known as "peach" secured from the laboratory of Dr. Erwin F. Smith. I also used different microtechnical methods supplementing the Flemmings' solution with the alcohol-acetic acid-formalin and Petrunkewitsch fixing solutions and staining with iron-alum haematoxylin in addition to the Flemmings' triple strain.

The Method and Path of Migration

The young tomato stem has the following tissues: a single-layered epidermis, a sub-epidermal, chlorophyllose parenchyma tissue from two to four cells in thickness, a collenchyma four or five cells in thickness, an inner cortex, a narrow vascular region consisting of bi-collateral bundles with scattered anastomosing phloem strands, and finally a relatively extensive central pith made up of large parenchyma cells with large elongated intercellular spaces which are of triangular shape in the transverse section. When considering the migration of *B. tumefaciens* in the stem of the tomato, three regions may be considered: the pith, the subepidermal tissues, and the xylem, if the latter is injured.

When the inoculating needle bearing a quantity of *B. tumefaciens* penetrates the young stem, a considerable number of cells of the various tissues of the host are destroyed. Microscopic examination of sections of the stem through the punctured region reveals every gradation of injury to the cells on the margin of the wound from lack of injury to total destruction. The bacteria are to be found growing in the mass of injured tissue. Most of the sections studied showed that the bacteria were growing in the tissues and not merely occupying the cavity formed by the needle. Very rarely the cavity resulting from the puncture was found to be filled with bacteria. Cells on the margin of the wound which were slightly ruptured but not completely disorganized by the passage of the needle were almost invariably found filled with bacteria within one hour after inoculation.

I found that the bacteria migrate as zoogloecae penetrating the pith and the sub-epidermal tissue (Plate XII, A, B, and C). The zoogloecae form in the mass of cells which were injured by the puncture of the needle. They then move vertically through the intercellular spaces. I was able to trace them through the pith both upward and downward from the injured cells around the puncture. In places in pith tissue some slight radial and

tangential migration was found. In the intercellular spaces of the pith I found elongated zoogloae perfectly continuous for distances from 0.35 mm. to 0.56 mm. and continuous but for slight interruptions for much greater distances. These zoogloae were found in fairly large numbers. My observations lead me to conclude that the zoogloae arise in the injured tissue in the region of the puncture and penetrate the intercellular spaces as isolated individual masses. The zoogloae rarely occur as an anastomosing system in the tissues of the pith. Branched zoogloae occur in the sub-epidermal region, where the anatomical structure is favorable for such development. The measurements given in a following section are based upon more than 80 distinct zoogloae. In some instances a single zoogloea passes through an intercellular space in a given region of pith. In others a whole group of zoogloae occurs in a restricted area. In such an area bacteria are in contact with some of the cells at every possible point since the zoogloae may occupy almost all the intercellular spaces in a restricted area. The zoogloae do not always completely occupy the intercellular space, but in some places they do completely occupy it and there they assume its shape.

Some of the physical properties of the zoogloae may be inferred from a study of their appearance and behavior. The zoogloae consist of a mass of bacteria imbedded in or surrounded by a jelly-like matrix. Apparently the form of these masses in tissue is dependent upon the size, shape, and general structure of the intercellular spaces in which they chance to be growing (Plate XII, A). Zoogloae growing in the long narrow intercellular spaces of the pith of the tomato stem assume their shape and appear as long narrow strands (Plate XII, C). Zoogloae growing in the sub-epidermal tissues assume a variety of forms. Most of the bacterial masses in the latter tissue are short and broad, and, where the shape of the intercellular spaces is favorable for such development, branching zoogloae may be found.

In *B. tumefaciens* the matrix surrounding the densely crowded bacteria appears to be of a fluid nature. The margins and advancing tips of the zoogloae are typically blunt and rounded in a convex manner with the outline sharp and clear (Plate XII, B and C). The form of the tip of the zoogloae resembles the meniscus of a heavy fluid such as mercury. The form of the tip of the zoogloae in the narrow intercellular space indicates that the matrix is of some substance not adhering to the walls of these spaces (Plate XII, B and C). The consistent progress of the zoogloal masses in the intercellular spaces at successive intervals indicates that the physical nature of the matrix allows a free flowing movement. In tomato tissue the matrix of the zoogloea of *B. tumefaciens* is not deeply stained

with the Flemmings' triple stain although the bacteria and the margins of the matrix are clearly differentiated.

Migration of the bacteria through the uninjured tissues of the stem is by way of the intercellular spaces of the pith and the sub-epidermal tissues. In no case was I able to recognize bacteria within any uninjured cell. In the young tomato stem the optimum path of migration of *B. tumefaciens* is through the large intercellular spaces of the pith. The bacteria penetrate to a greater distance from the puncture through the pith than through any other kind of uninjured tissue. Migration in the pith region is almost entirely vertical with very little migration in a radial or tangential direction. In the tomato stem the layer of chlorophyllose parenchyma from two to four layers of cells in thickness and occupying a position between the epidermis and the collenchyma, is also readily penetrated. This tissue is composed of loose parenchyma cells with many large irregularly shaped intercellular spaces. It resembles the spongy mesophyll of a leaf. The invasion of this sub-epidermal tissue occurs early, within the first two hours following inoculation, and results in large masses of bacteria widespread through the large intercellular spaces (Plate XII, A). My observations, however, led me to believe that the bacteria do not penetrate so far from the puncture in this region as in that of the pith. Migration is vertical, radial, and tangential in the sub-epidermal region.

The failure of the bacteria to migrate through the collenchyma tissue is probably due to the absence of suitable intercellular spaces. Migration through the cortex and through the vascular region was also found to be rare and possibly does not occur in early stages. The intercellular spaces of the cortex are much smaller than those of the pith region, and those of the vascular tissues are still smaller than those found in the cortex. Since the early migration of the bacteria is confined to the pith and the sub-epidermal region, where relatively large intercellular spaces are found, it seems possible that the size of the intercellular spaces may be the factor determining the optimum path of migration. Migration of the bacteria through the xylem vessels was found to be of rather common occurrence but seemed to be confined entirely to those vessels which had been injured. Apparently the xylem is penetrated by the bacteria only when injury to some of the elements of this tissue has made an opening for the parasites, for the bacteria were only found within vessels which had been broken by the insertion of the inoculating needle through a bundle. Under such conditions the bacteria were found in the vessels both above and below the puncture, indicating that the migration was independent of the flow of sap in the vessels. This path of migration is not so frequently used as that through the intercellular spaces of the pith and the sub-epidermal tissues.

The Rate of Migration

In order to obtain some information concerning the rate of migration of the bacteria, I measured the maximum distance to which the zoogloae had penetrated the intercellular spaces of the pith during various regular periods of time following the inoculation. The distance measured extended from the margin of the puncture to the farthest portion of the zoogloal strand to be observed in the longitudinal section. A total of 83 such measurements was made on material secured from 15 minutes to 3 hours after inoculation. The progress of the bacteria, therefore, was measured in the early stages only, and I have no data on the rate of migration in later stages. All zoogloae measured were located in the intercellular spaces of the pith. The material from which measurements were made had been grown at summer temperature in greenhouse and hotbed.

The migration of the bacteria through the intercellular spaces as zoogloae is almost unbelievably rapid, though not as rapid as the motion of individual bacteria as determined by Riker (4). Migration was found to be well under way within 15 to 30 minutes after the bacteria were introduced into the stem, and far advanced within an hour. Table 1 shows the results of the measurements.

TABLE 1.—*The penetration of intercellular spaces by zoogloae of Bacterium tumefaciens at different times after inoculation.*

Time (hours)	No. measurements	Average distance (mm.) penetrated by the zoogloae	Rate a minute in mm.
$\frac{1}{4}$	15	0.6008	.040
$\frac{1}{2}$	23	1.02	.034
1	6	1.3368	.022
$1\frac{1}{2}$	19	1.8178	.020
2	6	1.839	.015
3	14	2.6625	.015

The greatest distance traversed was 5.25 mm. in 3 hours, which is at a rate of 0.029 mm. a minute. The most rapid rate, however, is in the very earliest stages. The average rate for the first 15 minutes of 0.04 mm. a minute is almost twice as fast as the maximum rate for 3 hours. A growth curve⁴ with the average measurements plotted against the time helps in visualizing the rate of growth (Fig. 1).

⁴ I am indebted to Howard L. Clark for the graph illustrating the rate of growth of the zoogloae.

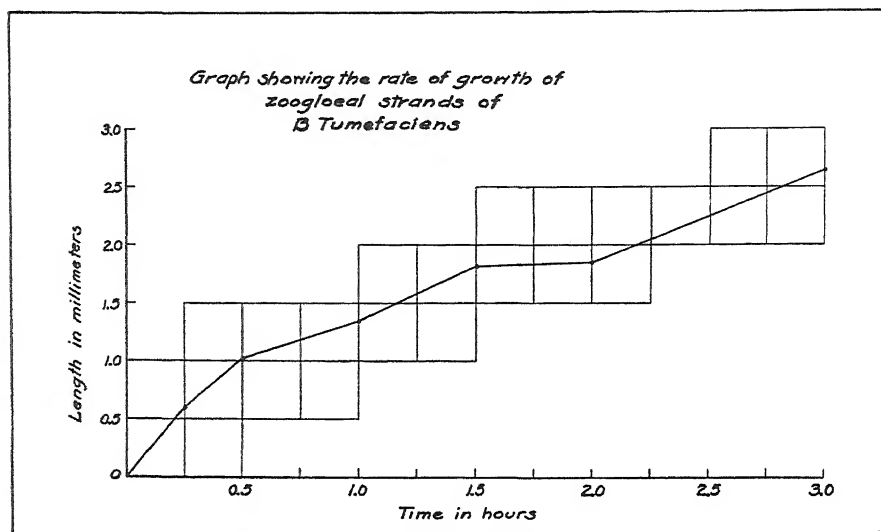


FIG. 1. Graph showing the penetration of intercellular spaces by zoogloae of *B. tumefaciens* at various times after inoculation.

RESULTS OF CHECKS

As indicated in a former section, I carefully checked all phases of this investigation from material obtained in 1927. The results from the check material confirmed the results obtained in the tomato. I found zoogloae in the intercellular spaces of both castor bean and tobacco tissue. The rates of migration of the zoogloae were of the same magnitude as those measured in tomato.

DISCUSSION

The conception of the migration of the bacteria through the intercellular spaces as zoogloae differs radically from the earlier ideas of Smith (11) who regarded the pathogen as an intracellular parasite whose migration was accomplished by the division and proliferation of the parasitized cells. It has been clearly shown by Riker (4), Robinson and Walkden (7), and by my investigation that *B. tumefaciens* occurs in the intercellular spaces. It is evident from my observations of the early stages of the migration resulting from inoculation that, after a few hours following their introduction into the tissues, the bacteria can be recognized only as widely scattered clumps clinging in the intercellular spaces. This is due to the fact that the rate of growth is very rapid and that after a few hours the tips of the zoogloae pass through the tissues leaving occasionally a few scattered clumps of bacteria. Under these conditions it is very difficult to determine

the method of migration. This accounts for the difficulties experienced by previous investigators in finding the migrating bacteria.

The rate of migration of *B. tumefaciens*, or actually the rate of growth of the zoogloae of this species, is so rapid that very early stages can be observed only in material killed within one hour after the bacteria have been introduced into the tissues. In the light of information concerning this rate, it becomes evident that the difficulty of the earlier investigators in tracing the first stages of migration was due to the fact that the material studied was too frequently taken long after the bacteria had passed far out into the tissue. An appreciation of the rate of migration of these bacteria indicates a change in technique.

The question really at issue here, then, is as to the medium in or through which the bacteria migrate from the puncture into the uninjured tissue. My own observation of numerous zoogloae passing along through the intercellular spaces confirms those of Robinson and Walkden (7) as to the manner in which *B. tumefaciens* migrates through the tissues. No other method of migration of these bacteria has been reported as actually observed by any investigator. The conception of the migration of the bacteria as zoogloae differs fundamentally from the idea of their migration as apparently implied by Riker (4), and (5), who throughout his discussion refers to the intercellular spaces as filled with liquid which furnishes a medium for the passage of the bacteria through the tissues. A realization of the difference between these two conceptions of the methods of migration is of fundamental importance in gaining an adequate conception of the behavior of *B. tumefaciens* in plant tissue. The importance of the conception of the migration of the bacteria as zoogloae as against the earlier theories cannot be overemphasized. It is related to these as fact to hypothesis.

SUMMARY

1. This investigation shows that *B. tumefaciens*, the pathogen of crown gall, migrates as zoogloae through the intercellular spaces of the sub-epidermal layer and those of the pith in young tomato plants.

2. The rate of growth of the zoogloae through the intercellular spaces is from 0.029 mm. to 0.04 mm. a minute, the more rapid rate occurring in the early stages of the migration following the introduction of the bacteria into the young tomato stems. The rate is most rapid for the first 30 minutes following inoculation. After 3 hours the rate is reduced one-half.

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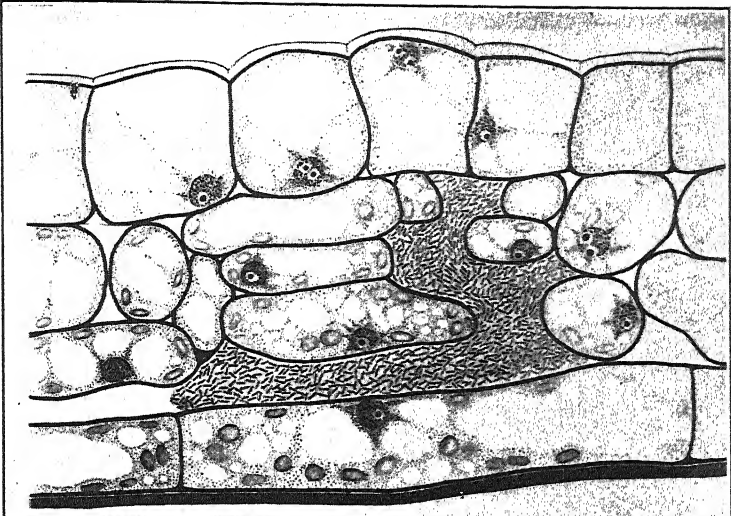
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EXPLANATION OF PLATE XII

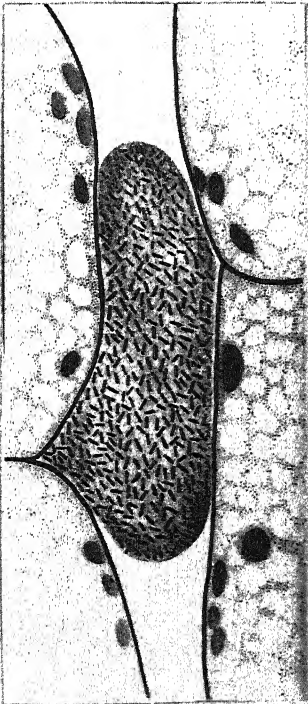
A. A radial longitudinal section through the outer portion of a tomato stem showing the relation of the zoogloae to the intercellular spaces of the sub-epidermal tissue. The sub-epidermal tissue in this region consists of three or four layers of chlorophyllose cells situated between the epidermis (shown above) and the heavy walled collenchyma (below). The zoogloea occupies and conforms to the characteristically irregular intercellular space. The rounded tip of the zoogloea seen at the left indicates the vertical direction of its movement. Nuclei, cytoplasm, and chloroplasts are illustrated in the cells. Magnification about 1250.

B. A tangential longitudinal section through the sub-epidermal chlorophyllose parenchyma of the young tomato stem. The zoogloea has reached its present location by moving in a radial direction from an adjoining intercellular space. The radial direction is perpendicular to the plane of the paper. The two rounded tips indicate that this zoogloea is advancing vertically in both directions. Cytoplasm and chloroplasts are shown in the cells adjacent to the intercellular space containing the zoogloea. Magnification about 1250.

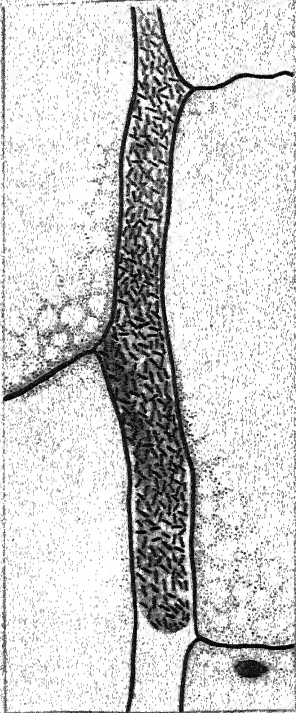
C. A longitudinal section through the central portion of the pith of a young tomato stem. An elongated zoogloea with the characteristically rounded tip is shown in the figure. As the zoogloea advances it assumes the shape of the narrow intercellular space which it occupies. Magnification about 1250.



A



B



C

SOME FUNGI OF THE STEMPHYLIUM TYPE AND THEIR RELATION TO APPLE ROTS¹

GEORGE A. NEWTON

In the Northwest the storage decays of apples are roughly classified by the market into the following five groups: blue mold, gray mold, anthracnose, false anthracnose, and *Alternaria* rot. It has long been known that the fungi causing the dark brown, or black, decayed areas roughly classified as *Alternaria* rot do not all belong to the genus *Alternaria*. To belong to that genus, according to Elliott (1), fungi must have the following characteristic essentials: "Conidiophores solitary or fasciculate, erect or subdecumbent, simple or branched, generally short, colored, conidia muriform, often with few longitudinal septa, ovate, obclavate or elongate, always with more or less definitely pointed apex, often long-beaked, colored, under favorable conditions forming chains." Spores from *Alternaria*-like cultures which are globular, sarcinaeform curved, or oblong, without apex or beak, he places in the genus *Stemphylium*.

With a view to studying in greater detail the different fungi causing apple rots in general, and the so-called *Alternaria* rot in particular, the fungi from decayed areas of several hundred apples from different localities of the state were isolated at the Washington Agricultural Experiment Station during 1925-26. The dark decayed areas referred to by the market in the Northwest as *Alternaria* rots yielded *Cladosporium*, *Phomopsis*, and *Stemphylium* besides *Alternaria*. The various isolations of *Stemphylium* were grouped in two types believed to represent species.

Two typical strains of rot-producing organisms were selected for special study. One of them (culture 4714-8B) produced abundant, colored, muriform conidia clustered on geniculate conidiophores, but never any perithecia; the other (culture 4716-6) produced conidia more sparingly. The conidia of the latter were colored, muriform, and usually borne singly on conidiophores that were slightly inflated at point of attachment to the conidium. In culture it usually produced from few to many perithecia, whose asci each contained eight muriform, yellow ascospores of the *Pleospora* type.

¹ Abridgement of a thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Agriculture at the State College of Washington.

The writer wishes to express his indebtedness to Dr. F. D. Heald, under whose direction this study was made, and to Professor B. F. Dana, for their helpful suggestions and inspiration throughout the work; also to Dr. Hannah C. Aase for her aid in the histological studies.

HISTORICAL REVIEW

In 1920 Horne (2) conducted an extensive investigation to determine the cause of spotting of apples in Great Britain. Among other fungi he found a species of *Pleospora* to which he gave the name *Pleospora pomorum*. He described the conidial form of this fungus as a *Stemphylium*.

Recently Rose and Butler (4) have isolated a species of *Pleospora* from decayed lemons from California, and from apples from Washington. They believe the fungus to be *Pleospora herbarum*, variety *citrorum*, and the conidial form they classified as a *Macrosporium*. They proved the pathogenicity of the fungus by cross inoculation.

RESULTS OF EXPERIMENTS

In the course of the experiments at Washington Experiment Station it was found that the *Pleospora* tended to lose its power to produce perithecia when carried long on rich media if frequently renewed. However, cultures made from a poured plate of ascospores that had not been renewed for several months would again produce perithecia (Fig. 4).

TABLE 1.—*The growth of cultures from single-spore isolations of Pleospora mali and Stemphylium congestum after seven days on various agars and at various temperatures*

Inoculum	Medium	Growth in centimeters at temperature of			
		30° C.	26° C.	20° C.	14° C.
<i>Pleospora mali</i>	2% dextrose potato agar	5.0	4.5	3.5	2.0
	2% dextrose beef agar	5.0	5.0	4.0	2.0
	Apple agar	4.0	6.0	4.0	2.0
	2% dextrose apple agar	6.5	6.5	4.0	2.0
	Prune agar	5.5	5.5	3.5	1.5
	Cornmeal agar	5.5	4.5	3.0	1.5
	Oatmeal agar	5.5	4.5	3.5	1.0
<i>Stemphylium congestum</i>	2% dextrose potato agar	5.5	3.5	2.5	1.5
	2% dextrose beef agar	4.5	3.5	3.0	1.5
	Apple agar	4.5	4.5	3.0	1.5
	2% dextrose apple agar	5.0	5.0	4.0	1.75
	Prune agar	3.5	4.0	3.0	1.5
	Cornmeal agar	3.0	3.0	3.0	1.5
	Oatmeal agar	6.5	6.0	4.0	1.5

Single spore isolations were made from both strains by means of the micro-loop. A small tube of glass was heated and so stretched and twisted as to form a microscopic loop. A suspension was made of spores to be isolated, and a small drop of the suspension placed on a sterile slide under the microscope. By use of the microscopic loop, single spores were picked out and planted in prepared plates of sterile potato dextrose agar.

Plantings taken from the single-spore isolation cultures and placed in various media at various temperatures showed some differences in amount of growth and in general appearance. (See tables 1 and 2 and figures 1 and 2.)

It is remarkable that at room temperature the cultures on prune and cornmeal agar produced perithecia within the week. All produced perithecia within 28 days, although all perithecia were not fertile at that

TABLE 2.—*The production of perithecia and the appearance of cultures from single-spore isolations of Pleospora mali and Stemphylium congestum after seven days on various agars at room temperature*

Inoculum	Medium	Perithecia	General appearance
<i>Pleospora mali</i>	2% dextrose potato agar	None	Gray, fluffy on top, medium density; dark underneath.
	2% dextrose beef agar	do	Almost white on top, felt-like; yellowish and black underneath.
	Apple agar	Few	Gray, fluffy on top, thin growth; gray underneath.
	2% dextrose apple agar	None	Gray, fluffy, whitish center on top; gray underneath.
	Prune agar	Present in all plates	Gray, thin growth on top; gray underneath.
	Cornmeal agar	do	Dark-gray, thin growth on top; dark-gray on bottom.
	Oatmeal agar	None	Gray, whitish center, dense on top; dark-gray on bottom.
<i>Stemphylium congestum</i>	2% dextrose potato agar	None	Black, dense growth on top; black underneath.
	2% dextrose beef agar	do	Black, dense growth on top; black and yellowish underneath.
	Apple agar	do	Dark, medium density on top; dark underneath.
	2% dextrose apple agar	do	Dark, medium density on top; dark underneath.
	Prune agar	do	Dark, medium density on top; dark underneath.
	Cornmeal agar	do	Dark, thin growth on top; dark underneath.
	Oatmeal agar	do	Black, dense growth on top; black underneath.

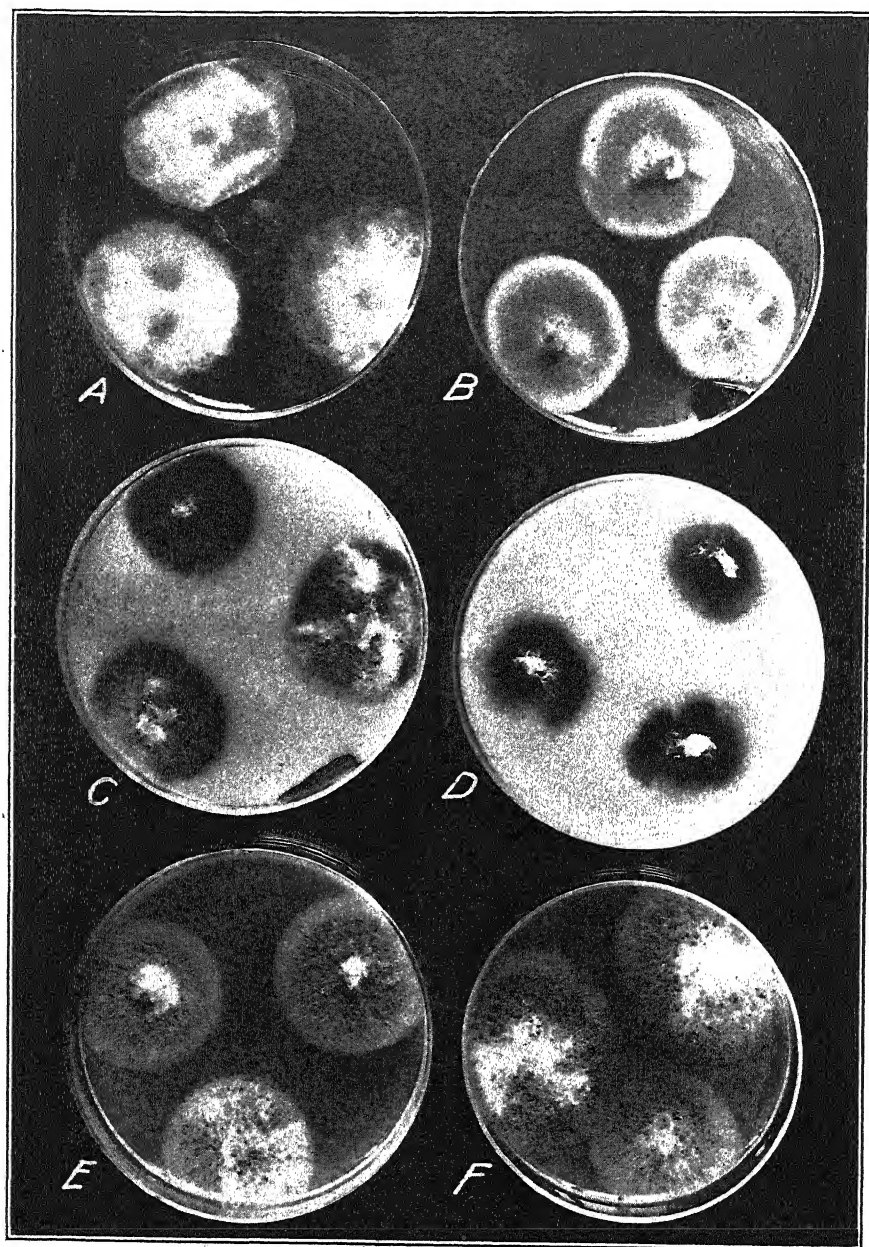


FIG. 1.—Plate cultures of *Pleospora* 4716-6 (*Pleospora mali*) from single conidio-spore isolation culture. Grown eight days at temperature of 20° C. A, Apple agar; B, prune agar; C, cornmeal agar; D, oatmeal agar; E, potato dextrose agar; F, beef bouillon dextrose agar.

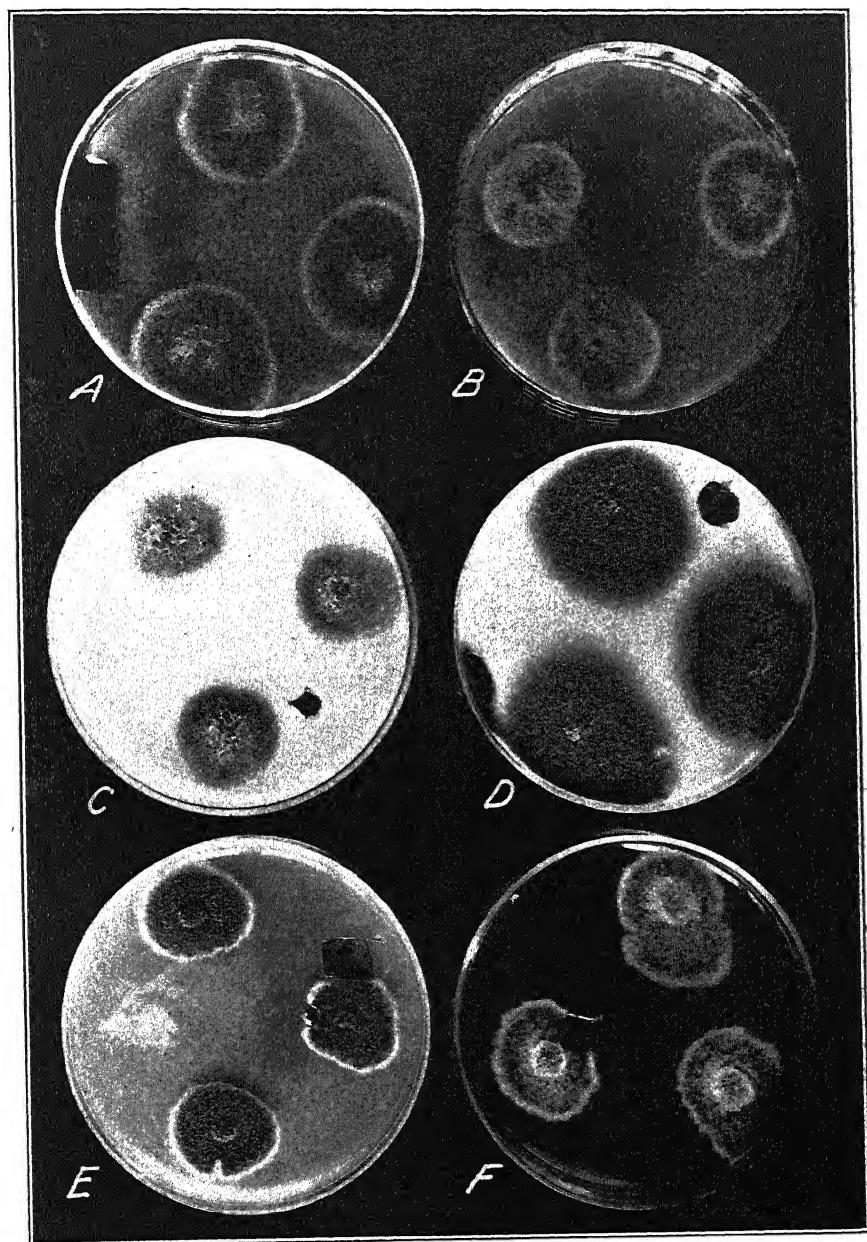


FIG. 2.—Plate cultures of 4714-8B (non-peritheciium—producing *Stemphylium*) from single spore culture. Grown for the same length of time, and at the same temperature as the cultures in figure 1. A, Apple agar; B, prune agar; C, cornmeal agar; D, oatmeal agar; E, potato dextrose agar; F, beef bouillon dextrose agar.

time. At the end of 48 days nearly all cultures held at 15° C. and 21° C. had produced fertile perithecia. (See tables 3 and 4.)

TABLE 3.—*The production and condition of perithecia in cultures of Pleospora mali (culture 4716-6) grown on various media and at different temperatures for 30 days*

Medium	Quantity and condition of perithecia at temperatures of			
	15° C.	21° C.	27° C.	30° C.
Cornmeal agar	Many; sterile	Many; fertile	Many; sterile	Very few; sterile
Apple agar	Few; sterile	Few; sterile	Very few; sterile	Very few; sterile
Prune agar	Few; sterile	Few; sterile	Few; sterile	Few; sterile
2% dextrose potato agar	Very few; sterile	Few; sterile	Very few; sterile	None
2% dextrose apple agar	Very few; sterile	Few; sterile	Very few; sterile	Very few; sterile
2% dextrose beef agar	Very few; sterile	Small, very few; sterile	Small, very few; sterile	None
Oatmeal agar	Very few; sterile	Few; fertile	Few; sterile	None

TABLE 4.—*The production and condition of perithecia in cultures of Pleospora mali (culture 4716-6) grown on various media and at different temperatures for 48 days*

Medium	Quantity and condition of perithecia at temperature of	
	15° C.	21° C.
Cornmeal agar	Many; fertile	Many; fertile
Apple agar	Few; fertile	Few; sterile
Prune agar	Few; fertile	Few; sterile
2% dextrose potato agar.....	Very few; fertile	Very few; fertile
2% dextrose apple agar	Very few; sterile	Few; sterile
2% dextrose beef agar	Very few, small; sterile	Few; sterile
Oatmeal agar	Very few; fertile	Few; sterile

The results of inoculating Jonathan apples with the single-spore isolation cultures are given in table 5, and also shown in figure 3. Comparison is here made with *Alternaria* and *Penicillium* as agents of apple decay. The *Penicillium* causes the most rapid decay at the lower temperatures and at room temperature, but at 30° C. *Alternaria* causes the most rapid decay. There is not much difference in the rate of decay caused by the *Pleospora* and the *Stemphylium*. The results of keeping inoculated Rome apples at temperatures ranging around the freezing point are given in table 6.

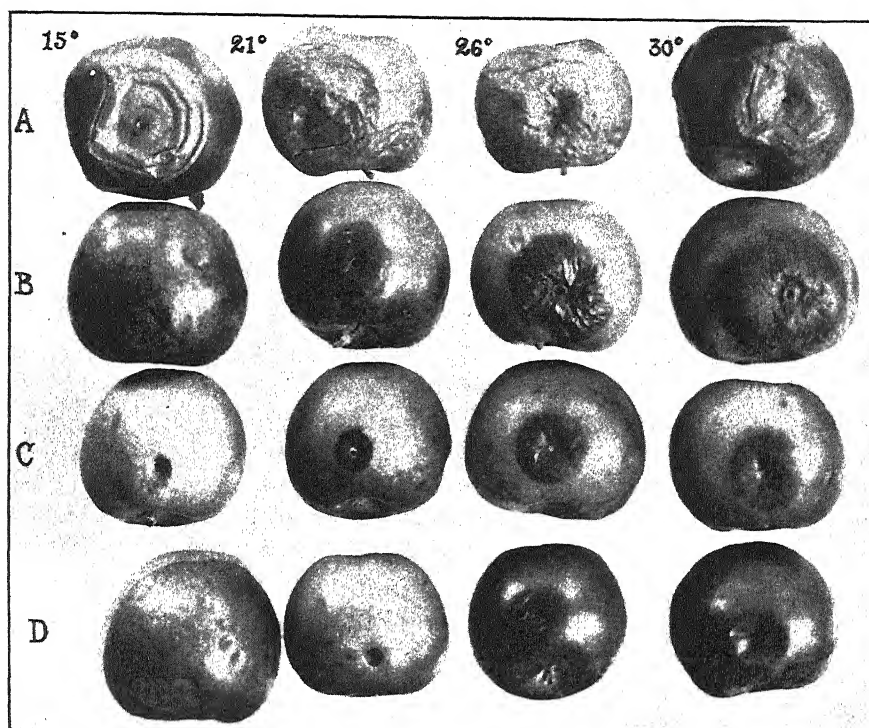


FIG. 3.—Jonathan apples inoculated March 25, 1927, with (A) *Penicillium*, (B) *Alternaria*, (C) *Pleospora* (4716-6), and (D) *Stemphylium* (4714-8B); and kept for two weeks at 15°, 21°, 26°, and 30° C.

MORPHOLOGY OF PLEOSPORA MALI

Some perithecia containing asci, and growing on an artificial medium, were killed in a solution of acetic acid in alcohol and dehydrated with

TABLE 5.—The extent of decay in Jonathan apples two weeks after inoculation with single-spore isolation cultures of various rot-producing organisms

Inoculum	Diameter in centimeters of decayed area at temperatures of			
	15° C.	21° C.	26° C.	30° C.
<i>Penicillium</i>	5.0	6.0	Whole apple	5.0
<i>Alternaria</i>	2.0	2.75	4.5	6.0
<i>Stemphylium congestum</i> (4714-8B)	0.5	1.75	3.5	2.5
<i>Pleospora mali</i> culture 4716-6	0.75	1.5	3.5	3.0
Control	0.0	0.0	0.0	0.0

TABLE 6.—*The extent of decay in Rome apples kept in cold storage (-2° C. to 2° C.) for 75 days after inoculation with Pleospora or Stemphylium*

Inoculum	Diameter of decayed area in centimeters
<i>Pleospora mali</i> culture 4716-6.....	2.0
<i>Pleospora</i> culture 4716-7.....	2.0
<i>Pleospora</i> culture 4727-1.....	1.0
<i>Stemphylium congestum</i> (4714-SB).....	2.0
<i>Stemphylium</i> culture 4734-E.....	2.5

alcohol. The material was imbedded in paraffin, using chloroform as a paraffin solvent. The perithecia were then sectioned, stained, and studied.

The perithecia are sub-emergent bodies more or less spherical, though often pear-shaped (Fig. 6). Each perithecium contains numerous asci, as many as 40 asci having been counted in some. The asci range in size from 146 to 200 microns long and from 26 to 29 microns wide, averaging 173.0 by 27.5 microns. Each ascus contains eight ascospores usually arranged in two rather irregular rows, though sometimes in a single row. The ascospores are yellow, later turning darker, muriform, usually six or seven-septate, often constricted in the middle. They have one to two longitudinal

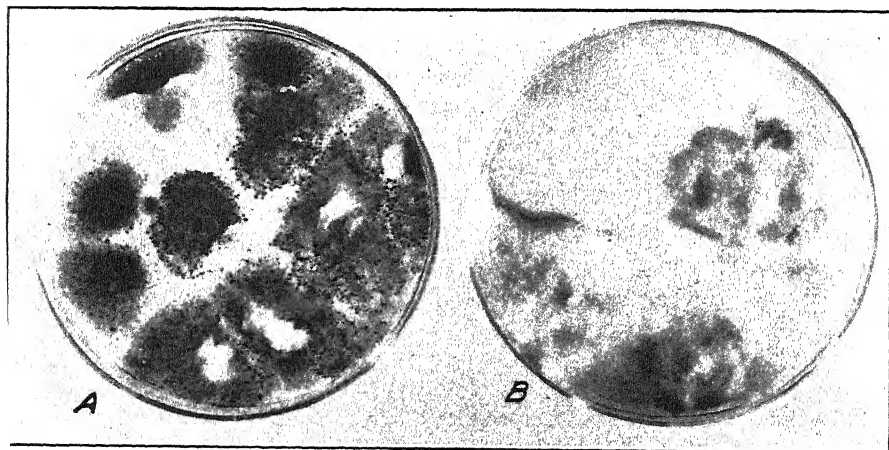


FIG. 4.—Plate cultures of 4716-6 and 4753-E2B, grown on prune agar at room temperature. Both are the perithecium-producing or *Pleospora* type. A, from a single spore isolation from conidia produced on a poured plate of ascospores from old dry plate culture that had not been renewed for several months. B, from stock culture carried about two years in ice box on potato dextrose agar changed about once a month. Though both were originally perithecium-producing, only the one from the single spore isolation from the cold culture now produces perithecia.

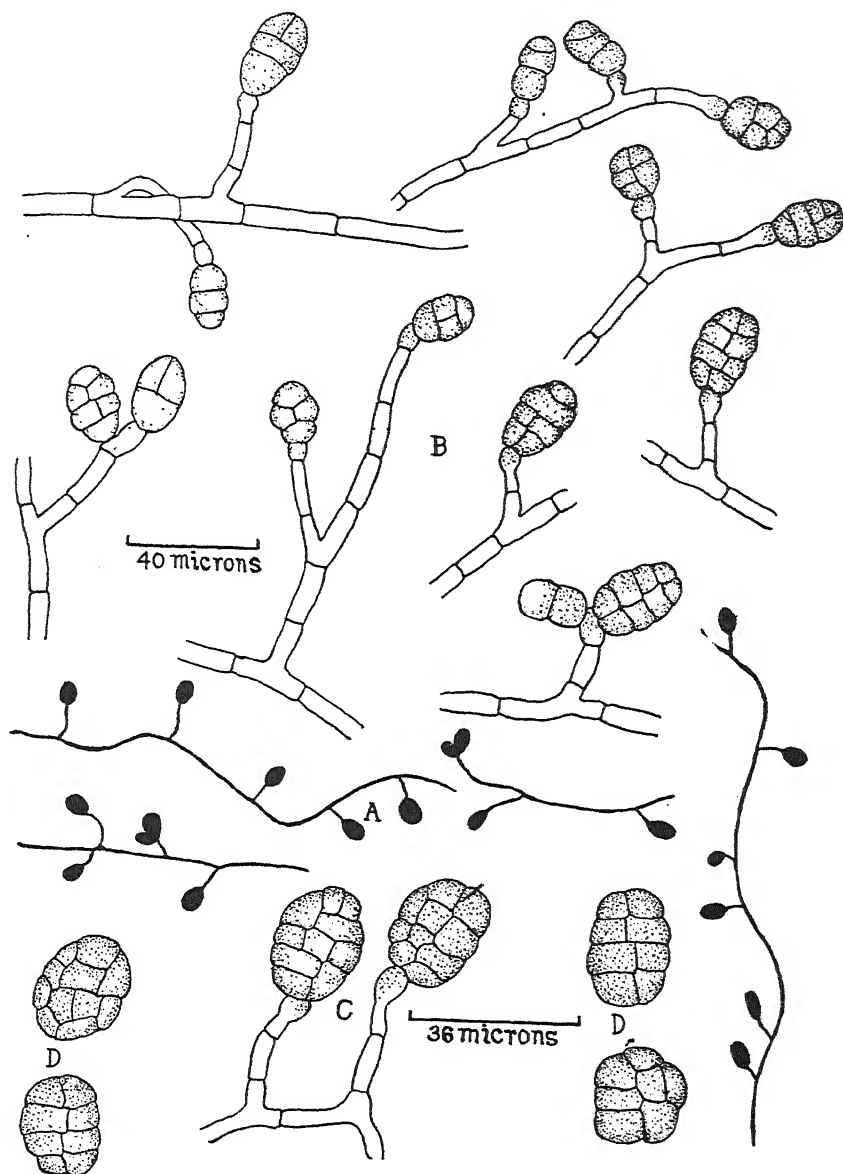


FIG. 5.—*Pleospora mali*. A, diagram to show arrangement of conidia and conidiophores; B, camera lucida drawing of formation of conidia on potato dextrose agar in Van Tieghem cell; C, camera lucida drawing of formation of conidia in plate culture of potato dextrose agar; D, camera lucida drawing of separate conidia.

septa, but these seldom extend the full length of the spore. They range in size from 28 to 34 microns long and from 11 to 14 microns wide, averaging 31.0 by 12.5 microns. The hyphae in artificial media often have a tendency to gather into rope-like strands.

The conidia are muriform, often warty, yellow, turning black, borne on conidiophores that are usually inflated at point of attachment to the conidium (Fig. 5). They range in size from 21 to 36 microns long and from 12 to 24 microns wide, averaging 28.5 by 18.0 microns.

Only sterile perithecia were found in lesions on apples which were infected naturally or artificially. Some of these were killed, fixed, embedded, and sectioned as described above in the case of the perithecia from cultures. They were of the same general shape as those grown in artificial media. In general they were located just underneath the epidermis of the fruit (Fig. 6). The presence of these minute bodies just underneath the epidermis gives a pimpled appearance to the surface of the cuticle. Some perithecia, however, are found deeply imbedded within the fruit.

Cross sections of the decayed area of the apple show the mycelium to be intercellular, though some bits of mycelium were found within the cells.

TAXONOMY OF *PLEOSPORA MALI* AND *STEMPHYLIUM CONGESTUM*

The perithecium-producing forms of *Stemphylium* which have been studied at the Washington Agricultural Experiment Station are clearly referable to *Pleospora*. The forms studied appear to be very closely related to *Pleospora pomorum* Horne, which has been isolated from spotted apples in England (2). The only other species of *Pleospora* reported on apples, *Pleospora herbarum*, var. *citrorum*, is too imperfectly described to permit of a certain differentiation, but for the present it seems distinct (Rose and Butler, 4).

Our species differs especially from *P. pomorum* in the size of the asci and ascospores, and in size and form of the conidia.

Pleospora pomorum

Asci—23 by 160–220 microns

Ascospores—golden—31–40 by 10.3–15.5 microns

brown—28–34.5 by 13–14 microns

Conidia—23–38.5 by 13.5–23 microns

Pleospora mali

Asci—26–29 by 146–200 microns

Ascospores—28–34 by 11–14 microns

Conidia—21–36 by 12–24 microns

The conidia of our species, which are also of the *Stemphylium* type, are more elongated than the sphaero-quadrilateral type figured by Horne and Horne (3).

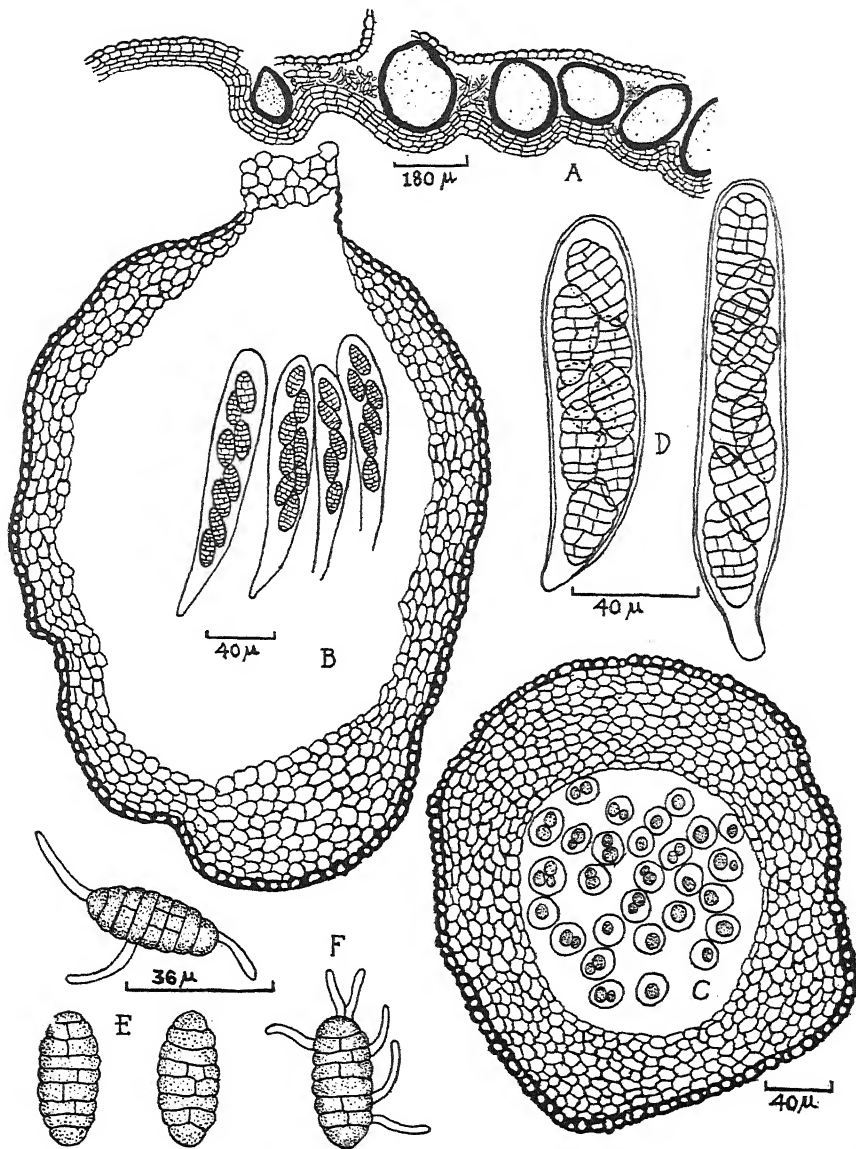


FIG. 6.—*Pleospora mali*. A, semi-diagrammatic drawing of perithecia underneath epidermis of apple; B, longitudinal section through peritheciium grown in potato dextrose agar; C, cross section through peritheciium grown in potato dextrose agar, showing cross sections of asci and ascospores; D, asci containing ascospores from perithecia grown on potato dextrose agar; E, free ascospores; F, germination of ascospores after 6 hours in Van Tieghem cell on potato dextrose agar.

The following is a technical description of the *Pleospora* which has been found associated with, and causing decay in, Washington apples.

Pleospora mali nov. sp.

Hyphae variously branched, septate, at first pale, but becoming dark, showing a tendency to be grouped in strands in cultures; perithecia none to few or very numerous in the various strains in culture, and varying in size from minute, imperfect forms to 1 mm. in diameter; perithecia on the fruit of the apple, immersed becoming erumpent, always infertile, sometimes deep below the epidermis, but generally subepidermal; asci cylindrical, straight, 8-spored, $146-200 \times 26-29 \mu$ or average $173 \times 27.5 \mu$; ascospores ovate-oblong, muriform, subbiseriate, 7-septate, with 1 to 2 longitudinal walls, orange to brown, $28-34 \times 11-14 \mu$ or average $31.0 \times 12.5 \mu$; conidia of *Stemphylium* type, few or numerous, terminal on short or elongated, septate conidiophores which are generally expanded below the spore; conidia muriform, fuscous to very dark, ovate-oblong to irregular or sometimes nearly sphaero-quadrilateral, 1-3 transverse septa, 1 longitudinal septum, wall smooth when young, becoming tuberculate with age, $21-36 \times 12-24 \mu$ or average $28.5 \times 18 \mu$. Isolated from lesions on Rome Beauty, Jonathan, and Yellow Newtown apples, and determined by pure culture inoculations to cause a firm brown rot of the fruit.

The non-perithecium-producing forms which have been studied in this paper produce conidia of the *Macrosporium-Stemphylium* type in great abundance. They are clearly distinct from the perithecium-producing forms, as shown by cultural characters and the manner of conidial formation. The various isolations have shown a marked constancy in cultural behavior, always producing conidia in abundance on various types of media. It has not been possible to refer our species to any of the described species of the *Stemphylium-Macrosporium* type. It will be described as new.

Stemphylium congestum nov. sp.

Hyphae variously branched, septate, dark, making a dense growth on various media with very copious production of conidia; conidia muriform, sphaero-quadrilateral to ovate-oblong, 1 to 3 transverse septa, 1 longitudinal septum or none, smooth when young, but tuberculate with age, and becoming nearly black, $17-30 \times 12-19 \mu$, average $23.5 \times 15.5 \mu$; conidia produced acrogenously on simple or slightly branched septate conidiophores, never single but accumulating in botryose clusters of two to many. Isolated from lesions on Rome Beauty and Jonathan apples and determined by pure culture inoculations to cause a firm brown rot of the fruit.

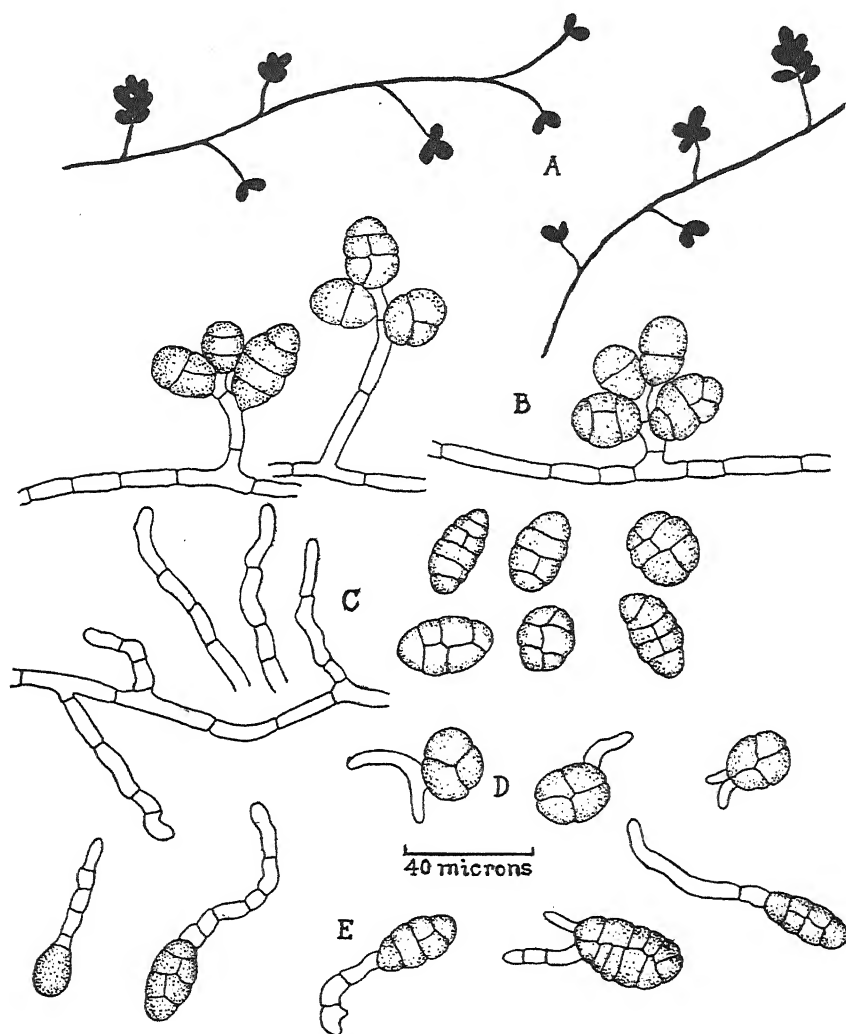


FIG. 7.—*Stenphylium congestum*. A, diagram showing arrangement of conidia and conidiophores; B, formation of conidia on potato dextrose agar in Van Tieghem cell; C, drawing showing conidia and conidiophores grown on potato dextrose agar plate; D, germination of conidia after 6 hours in Van Tieghem cell on potato dextrose agar; E, germination of conidia after 3 hours in distilled water.

SUMMARY

The black rots generally referred to by market inspection reports in the Northwest as "Alternaria rot" have been shown to be caused by several different fungi, among which are two different species producing conidia

of the *Macrosporium-Stemphylium* type. These two were selected for detailed study.

One of these is a perithecium-producing form which is here described as *Pleospora mali* nov. sp. The perithecia produced in culture media form mature asci and ascospores, while the perithecia produced on the fruit have never been found to contain mature asci nor ascospores.

The other of the two species selected for special study is a non-perithecium-producing form which is here described as *Stemphylium congestum* nov. sp. Its conidia are usually borne in dense clusters on the side and top of crooked conidiophores.

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NEW PHYSIOLOGIC FORMS OF *TILLETIA LEVIS* AND *T. TRITICI*¹

E. F. GAINES²

Stinking smut has long been one of the major diseases of wheat and second only to the rusts in its destructiveness. In the United States the amount of smutty wheat coming to market during recent years is cause for grave concern. In the Pacific Northwest as much as 50 per cent of the cars coming to the terminal shipping points are given a smut dockage.

When the Western agronomists assembled at Moro, Oregon, for their annual meeting, June 27, 1927, Superintendent D. E. Stephens pointed out that there was something new to be seen in the smut resistance plots. Selections of Turkey wheat that had been resistant in former years contained so much smut that one could scarcely find a good wheat head. Mr. Stephens had obtained the inoculum, presumably a mixture of *Tilletia tritici* and *T. levis*, with which the seed had been inoculated from the Grain Supervision Office of the Bureau of Agricultural Economics, at Portland, Ore. Even such wheats as Ridit, Albit, Martin, and White Odessa contained from 7.7 to 51.5 per cent of smutted heads (7). These wheats had been practically smut-free in similar tests for years. In contrast with this result at Moro, the same varieties inoculated with local *T. tritici* at Pullman, Washington, did not show a trace of smut. However, a 20-acre field of Albit wheat on the College Farm at Pullman did contain 0.2 per cent of smutted heads. Since the seed was smut-free the year previous and had been sown without treatment, and since Albit had not produced a trace of smut during the

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previous four years in the smut tests in which local *T. tritici* inoculum was used, it seems possible in the light of evidence hereafter given that smut which attacked Albit was blown in from the great wheat belt to the West and infested the soil. The smut which occurred in Albit proved to be three-fourths *T. levis* and one-fourth *T. tritici*.

The branch experiment stations at Lind and Waterville, Wash., west of Pullman, likewise reported alarming increases in the amount of smut appearing in hitherto resistant varieties. At Lind, a highly resistant strain of Turkey (Wash. No. 326) that had been sown on the station farm without seed treatment for 10 years with no appreciable smut infection produced 75 per cent of smutted heads in 1927. The seed of this crop was inoculated with smut from a field of this same strain of Turkey which had been grown on the station in 1924. This is considered evidence that a new form of bunt to which this strain of Turkey is susceptible has been present at least since 1924. A microscopic examination showed it to be *T. levis*, and not *T. tritici*, which had been the prevailing smut on the Pacific Coast in former years.

A farmer near Waterville, Wash., who had been growing a resistant strain of Kharkof obtained from Sherman County, Ore., in 1918, had never considered seed treatment necessary for this particular variety. In 1925 a few heads of smut appeared in his crop. In 1926 the smut was much worse. The farmer, supposing the increase due to the unusually favorable season for smut, again sowed his crop that fall without treating the seed. In 1927 his entire crop of Kharkov averaged 60 per cent of smutted heads. Soil infestation with *T. tritici* is common in that locality, and a susceptible variety of wheat would have smutted badly every year. It seemed logical, therefore, to assume that a new form of smut to which Kharkof is susceptible came in, rather than to assume a change in the host. In support of this hypothesis a smut sample from this field was identified as *T. levis*.

Mr. B. B. Bayles (1), of the Judith Basin Substation, Moccasin, Montana, likewise reported heavy smut infection on heretofore resistant strains. At Moccasin there was 14.2 per cent of smut on Ridit, and from 80 to 90 per cent on such varieties as Kanred, Minhardi, and Minturki. This is the highest percentage of smut yet reported on Ridit in America. The inoculum for this experiment was obtained from a field of Turkey near the station and has been identified as *T. levis*.

In extensive smut surveys from 1918 to 1921, the bunt occurring in the Pacific Northwest was almost without exception identified as *T. tritici*, and wheat breeding experiments to develop resistant varieties have been conducted using spores of this species of the smut organism as inoculum. The above observations show that the other species, *T. levis*, is now present, that this latter is more virulent than the common forms before present, and that it has been increasing and spreading during recent years. Whether this is a

new physiologic form of *T. levis* different from that occurring east of the Rocky Mountains remains to be determined. Indirect evidence indicates that it is, for tests at Kansas from 1921 to 1923 showed Kanred to be very resistant to the common *T. levis* of that state, while the test at Moccasin in 1927 showed Kanred to be very susceptible.

It is known that the resistant wheats of America sometimes produce considerable smut in tests conducted in Europe, although in most cases they maintain their relative resistance. Rodenhiser and Stakman (4) obtained collections of both *T. tritici* and *T. levis* from foreign countries in 1925 and compared their virulence on Kota, Marquis, and Einkorn. A collection of *T. tritici* from Norway was much more virulent than one from New Zealand. A collection of *T. levis* from Hungary was more virulent on Einkorn than a collection from Egypt, but less virulent on Marquis. Both were distinct from the local *T. levis* of Minnesota.

The Welch Plant Breeding Experiment Station at Aberystwyth, Wales, tested six American wheats from the Washington State Experiment Station in comparison with four common English varieties, with the results shown in table 1.

TABLE 1.—Comparative resistance to stinking smut of 6 American and 4 English wheats tested at Aberystwyth, Wales. Data furnished by Kathleen Sampson

Variety	Percentage of infected plants, 1926	Percentage of smutted heads	
		1925	1926
<i>American wheats</i>			
Florence	38	7	20
White Odessa	9	—	4
Ridit	2	3	1
Turkey	12	17	11
Hussar	0	0	0
Martin	0	0	0
<i>English wheats</i>			
Hen Gymro	83	67	81
Yoeman	63	—	54
Standard Red	80	—	78
Little Joss	56	—	47

The American varieties were much more resistant than the English, although White Odessa and Ridit, which so far have been smut-free in tests at Pullman, did produce a small percentage of smutted heads. Martin and Hussar were smut-free, as they have been at Pullman. Florence and Turkey contained slightly more smut than is normal in similar tests at Pullman, but still would be classed as resistant wheats in comparison with the English varieties.

TABLE 2.—*Comparative resistance to Tilletia tritici of 11 American wheats at Cambridge, England, 1924-1927. Data from W. A. R. Dillon-Weston, University of Cambridge*

Variety and source	Year tested	Percentage of bunted heads
Sherman C. I. 4430 from Moro, Ore.	1924	1
	1925	1
	1926	2
	1927	8
	1924	7
Turkey C. I. 1558 from Manhattan, Kan.	1925	53
	1926	51
	1927	74
Kanred C. I. 5146 from Moe-casin, Mont.	1924	10
	1925	27
	1926	18
Clackamas C. I. 6241 from Davis, Calif.	1925	8
	1926	15
	1927	56
Masters Perfection C. I. 4726 from Chico, Calif.	1924	6
	1925	4
	1926	23
	1927	59
	1924	2
White Odessa C. I. 4655 from Moro, Ore.	1925	0.3
	1926	0
	1927	9
	1924	trace
Ridit C. I. 6703 from Moro, Ore.	1925	0.7
	1926	1
	1927	4
	1924	0
Martin C. I. 4463 from Moro, Ore.	1925	0
	1926	0
	1927	0.5
	1924	0
Hussar C. I. 4643 from Moro, Ore.	1925	0
	1926	0.2
	1927	0.6
	1925	26
Fulcaster C. I. 6162	1926	89
	1925	0.5
Berkeley Rock	1926	1
	1927	2

At Cambridge, England, 11 varieties of American wheat were tested during the four years 1924 to 1927. The results are shown in table 2.

Sherman, White Odessa, Ridit, Martin, Hussar, and Berkeley Rock, which seldom showed a trace of smut in American tests, produced from 0.5 to 9 per cent of smutted heads. They were much more resistant, however, than the others, especially in 1926 and 1927. The increase in 1927 suggests either a very favorable season for smut or the introduction of a more virulent form. A test with *T. levis* in 1924 on four of the varieties, Sherman, Ridit, Martin, and Hussar, indicated little, if any, difference in its pathogenicity from that of *T. tritici*.

In 1925, Dr. Theodore Roemer visited the United States and arranged a cooperative experiment in which smut and wheat were exchanged between the University of Halle-Saale, Germany, and the State College of Washington at Pullman. The results at Pullman (2) in 1926 are shown in table 3.

TABLE 3.—A comparison of German and American smut (*T. tritici*) in respect to infecting power on eight local Pacific Northwest wheats and four wheats from Halle-Saale, Germany, at Pullman, Washington, in 1926

Variety	Source of Seed	Source of inoculum and percentage of bunted heads	
		America	Germany
Hybrid 128	Pullman, Washington	92	98
Triplet	do	79	84
Little Club	do	91	100
Turkey (Wash. No. 326)	do	2	36
Martin	do	trace	38
Ridit	do	0	1
Hussar	do	0	19
White Odessa	do	0	71
Hohenheimer (Behaart)	Halle-Saale, Germany	36	1
Hohenheimer 77 (Unbehaart) ..	do	33	0
Fürst Hatzfeldt	do	54	15
Heils Dickkopf	do	42	0

The preliminary experiments at Pullman in 1926 left no doubt as to the different physiologic specialization of the two collections of *T. tritici*. The German smut in every case was more virulent on the American wheats but less so on the German wheats. Of the 12 wheats tested, Ridit was the only one that was very resistant to both forms. White Odessa and Heils Dickkopf were the best differential testers. Each was immune from its native smut but produced 71 and 42 per cent, respectively, of smutted heads from the foreign inoculum.

Dr. Roemer enlarged his experiment at the University of Halle in 1927 to include smut collected from the various sections of Germany where wheat is grown, as well as samples from Hungary, Switzerland, Holland, Sweden, Denmark, and the United States. He used as hosts three resistant and two susceptible German wheats and five resistant American wheats; also three hybrid populations of a resistant-susceptible cross. Each test was replicated four times. There were 100 seeds sown in each row, making 400 seeds for each test, from which an average of 360 plants was obtained. The entire experiment of 13 varieties treated with smut from 15 sources represented 780 rows with a total of 70,200 plants. With Dr. Roemer's permission the data are given in table 4 in terms of smutted plants, including the plants containing both wheat and smut as well as those entirely smutted.

The inoculum from each locality was good, viable material, as is shown by the uniformly high infection on the susceptible varieties, Stocken and Panzer. The smut from Switzerland was the least virulent form, while the one from Cosel was, as measured by the average infection of all varieties, the most virulent. The two varieties that showed considerable resistance to all forms were Hohenheimer 77 and Ridit. The smut from Cosel infected Hohenheimer 77 to the extent of 34 per cent of the plants, but none of the other smuts produced more than 9 per cent in this variety. The smut from Bonn infected Ridit more than any other collection—24 per cent. Heils Dickkopf, Tubeuf, and Hussar were also quite resistant, having failed to become infected by one or more of the collections, but producing as much as 50 to 74 per cent when inoculated with spores from some one of the other sources. Martin, White Odessa, and Kharkov were more or less susceptible to some of the collections but resistant to others. The mixed populations of the resistant susceptible cross were intermediate, as might be expected, being most susceptible to Cosel smut and most resistant to the Hungarian smut, like Heils Dickkopf, the resistant parent.

In general, the smuts from Cosel, Breslau, Bonn, Weihenstephan, and Hohenheim, Germany, were the most virulent, those from Hungary, Göttingen, Germany, were intermediate, and the others were relatively weakly infective. The smut from Hungary attacked Martin and Kharkov more severely than any of the others, while the smut from Göttingen produced the highest infection of any on Panzer. Cosel smut was most severe for Heils Dickkopf and Hohenheimer 77. Tubeuf and White Odessa wheats were most severely infected with Breslau smut. Ridit was least resistant to Bonn smut, and Hussar least resistant to the smut from Hohenheim.

It would be premature to say on the basis of this experiment just how many physiologic forms are represented. The possibility of mixtures in both host and parasite is always a source of apprehension. There are five collections of stinking smut from which one or more of five different wheat

TABLE 4.—The percentages of smutted plants obtained in 10 varieties and 3 hybrid populations of wheat inoculated with spores of 14 different collections of *Tilletia tritici* and one collection (Arlington Farm) of *T. tritici*. Experiment conducted at Halle-Saale, Germany, by Theodore Roemer, 1927

Wheat varieties	Average															
	Cosel, Germany	Breslau, Germany	Bonn, Germany	Weihenstephan, Germany	Hohenheim, Germany	Hungary	Göttingen, Germany	Halle, Germany	Wageningen, Holland	East Prussia, Germany	Landskrona, Sweden	Lynghy, Denmark	Zurich, Switzerland	Pullman, Wash. U. S. A.	Arlington Farm, U. S. D. A.	Average
Hohenheimer 77	34	9	8	3	6	0	3	3	5	6	3	1	12	4	3	5.5
Riddit	7	10	24	10	14	5	3	5	6	3	1	2	0	0	0	6.4
Heils Diekkopf	50	15	46	29	16	0	8	8	9	9	8	3	10	6	12	15.3
Tubeuf	58	59	27	44	27	9	32	10	0	55	29	16	5	0	0	22.5
Hussar	50	46	26	32	74	57	25	1	0	8	1	19	0	1	9	23.1
Martin	77	61	64	70	72	78	27	1	1	26	3	20	12	1	10	34.9
Kharkov	52	62	38	60	38	69	51	40	52	27	34	23	44	15	37	43.8
White Odessa	83	85	84	67	80	79	74	32	7	33	2	16	40	8	38	48.5
Panzer	89	88	76	85	82	^a	91	83	83	84	87	79	^a	70	67	81.8 ^a
Stöcken	93	89	88	82	86	89	92	93	93	85	88	88	66	89	87	87.2
Heils Diekkopf	62	40	35	34	32	8	29	35	40	17	17	18	28	17	18	28.7
×	65	38	43	42	41	13	32	29	42	25	18	18	19	36	22	32.2
Hatzfeld	59	48	39	48	29	14	34	31	26	28	20	23	23	44	22	32.5
Average	59.9	50.0	46.0	46.6	45.9	35.1 ^a	38.5	28.5	27.8	28.4	23.8	25.8	20.7 ^a	22.8	25.3	

^a Percentage of smut in Panzer not shown for the collections from Hungary and Switzerland.

varieties are smut-free. Various hybrid combinations of the most resistant wheat varieties should give segregates more resistant than Ridit or Hohenheimer 77. Dr. Roemer, in his article (5), does not attempt to estimate the number of physiologic forms in his collections, but suggests that each collection is probably a mixture of several pure lines. He emphasizes the need of using the most virulent collections for testing hybrid populations in order to isolate the most generally resistant segregates.

There is considerable evidence to show that what may be more virulent physiologic forms have been spreading rapidly in the United States during recent years. Since 1924, stinking smut has been the most destructive parasite of wheat in America. The enormous quantity of smutty wheat coming to market has induced the larger terminal elevators of the Central West, according to Shollenberger (6), to install washers for removing the smut. Smut has been on the increase, especially in Kansas, Colorado, and Nebraska, where hard red winter wheat is grown, as well as in Virginia and Pennsylvania, where soft red winter wheat predominates. The losses in these five states alone in 1926, according to Kirby (3), was 10 per cent in Kansas, 8 per cent in Colorado, 6 per cent in Nebraska, 6 per cent in Pennsylvania, and 5 per cent in Virginia. Taking the total wheat produced in these five states according to the 1926 United States Department of Agriculture Year-book and multiplying by the percentages, the loss amounts to 20,659,460 bushels.

Kirby's estimates are corroborated in a general way by the percentage of carloads of wheat graded as smutty at the principal shipping posts, as shown in table 5.

Table 5 was compiled by the Office of Grain Investigations of the Bureau of Agricultural Economics. There are fluctuations from year to year, but the general trend is in the direction of increasing amounts of smut. The total average receipts at Kansas City, Omaha, Baltimore, and Philadelphia were 12, 16, 23, 26, and 28 per cent of smutty wheat for the five years 1923 to 1927. This is a consistent increase in which the amount has more than doubled in five years. In the Pacific Coast States, where seed treatment has been emphasized and where resistant varieties like Ridit are being introduced, there is a slight decline in the amount of smut since 1924. At Minneapolis in 1926 there was a sudden increase of cars grading smutty. This was confined almost entirely to the hard red winter and to the durum classes of wheat. Durum has always been considered resistant to smut but in 1927 at Minneapolis there were 22 per cent of the cars of durum, and 24 per cent of the cars of hard red winter wheat that graded smutty. The hard red spring class remained practically smut-free as in former years.

At Bozeman, Mont., there has been a rather rapid increase of smutty wheat from 12 per cent of cars grading smutty in 1923 to 47 per cent grad-

TABLE 5.—Percentage of cars of wheat received at different markets which graded smutty. The shipments from July 1 to June 30 of the following year were taken as the crop year. (Less than 1 per cent not marked). Compiled by E. G. Boerner and F. C. Meier

	1923	1924	1925	1926	July-October 1927
Astoria, Ore.	52	69	38	55	47
Baltimore, Md.	0.8	2	9	8	16
Bozeman, Mont.	12	19	17	34	47
Denver Colo.	21	25	36	25	19
Des Moines, Ia.	—	8	7	15	20
Detroit, Mich.	—	—	11	8	11
Duluth, Minn.	—	5	17	15	14
Indianapolis, Ind.	3	6	2	6	22
Kansas City, Kans.	8	11	10	22	14
Kearney, Neb.	4	35	54	28	12
Lawrenceburg, Ind.	6	37	38	44	19
Lincoln, Neb.	—	12	26	20	9
Los Angeles, Cal.	20	12	15	18	16
Louisville, Ken.	5	9	2	4	11
Minneapolis, Minn.	2.4	0.7	—	16	12
Ogden, Utah	14	21	29	33	31
Oklahoma City, Okla.	1	1	1	5	1
Omaha, Neb.	14	26	40	40	25
Philadelphia, Pa.	3	2	10	8	31
Portland, Ore.	45	60	30	42	40
Sacramento, Calif.	6	9	—	12	24
Seattle, Wash.	35	41	24	28	32
Stockton, Calif.	26	11	6	19	6
Tacoma, Wash.	42	54	30	35	32
Toledo, O.	1	3	2	2	11

ing smutty from July to October, 1927. The experiments at Moccasin, Mont., showing the presence of a strain of smut which attacks the hard red winter wheats with great virulence suggest a plausible explanation for the increase.

No doubt climatic conditions and cultural practices are responsible for wide fluctuations from year to year, but when the trend over a period of years is in the direction of increasing amounts of smut, and when carefully conducted experiments show forms of smut attacking types of wheat that were resistant in former years, the conclusion seems justified that the new forms of smut are responsible for some of this increase.

Tisdale, Leighty, and Boerner (8) have shown that *T. levis* is the most prevalent species east and south of the Dakotas, but since the prevalence of smut is increasing in these regions also, there is no reason to suppose that *T. levis* is less subject to physiologic specialization than *T. tritici*. In Dr. Roemer's experiments all forms were *T. tritici* except the smut from Arling-

ton Experiment Farm, Rosslyn, Va., which was *T. levis*. The smuts which attacked the Turkey, Kharkof, and other varieties so severely at Moccasin, Mont., Lind and Waterville, Wash., were all *T. levis*. The smut collected on Albit at Pullman, Wash., contained smooth and rough spores in the ratio of 3:1. The smut common to Pullman and the one that has been used in the breeding work of the Washington Station is *T. tritici*. In respect to the reaction on the resistant Turkeys, which probably represent a major part of the hard red winter wheat as a class, five forms may be assumed from analyzing the tests of the past two years. They may be listed as follows:

<i>Tilletia tritici</i>	Pullman form	Infects Turkey 5 per cent
do	German form	Infects Turkey 35 per cent
do	North Dakota form (6)	Infects Durum but not Turkey (?)
<i>Tilletia levis</i>	Old form	Infects Turkey 10 per cent
do	Eastern Washington form	Infects Turkey 75 per cent

This is probably a much simpler classification than subsequent experiments will show actually exist. The percentages of infection are purely hypothetical, since data under comparable conditions are not available. Experiments are in progress at several of the western experiment stations designed to give this information in 1928.

The most hopeful point of attack to meet this new situation is a greatly increased program of breeding for resistance to the new forms. In the meantime, increased emphasis needs to be placed on seed treatment and cultural practices to control or lessen the damage caused by stinking smut, the most destructive cereal disease in the United States.

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INFLUENCE OF ENVIRONMENTAL FACTORS ON THE SEASONAL PREVALENCE OF CORN SMUT^{1,2}

F. R. IMMER AND J. J. CHRISTENSEN³

INTRODUCTION

The influence of climatic factors on the development of corn smut, *Ustilago zae* (Beck.) Ung., and the relative susceptibility of corn plants at various stages of development is imperfectly known. In recent years, however, the relation of these factors to infection and prevalence of *U. zae* has received considerable attention.

Selby and Hickman (11), in 1897, concluded that corn smut was more prevalent under dry weather conditions. More recently, Potter and Melchers (10) and Coffman et al (4) have confirmed these observations.

Arthur and Stuart (1) and Piemeisel (9) concluded that humid weather was most favorable for smut infection. Potter and Melchers (10), on the other hand, did not believe that lack of moisture could be considered a limiting factor for smut infection, provided conditions were favorable to the growth of the host. Tisdale and Johnston (12) concluded from their greenhouse experiments that humid conditions favored infection. They were also of the opinion that high temperatures were conducive to infection, and that low temperature was one of the chief factors limiting infection.

Corn seedlings seldom become infected under natural field conditions. A number of investigators, however, have reported seedlings to be very susceptible when artificially inoculated. Tisdale and Johnston (12), by means of a hypodermic needle, injected young seedlings with a conidial suspension of *U. zae*, and concluded that seedlings seemed to be more susceptible than older plants. They stated that strains of corn reacted the same way in the field as they did in the greenhouse when artificially inoculated.

Immer (6) readily infected corn seedlings by hypodermic inoculation in the greenhouse but found no appreciable correlation between seedling

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² This study was made as a part of the general program of breeding for disease resistance being conducted cooperatively by the sections of plant breeding and plant pathology.

³ The authors wish to express their appreciation to Drs. H. K. Hayes and E. C. Stakman for help and criticism in preparing this paper.

susceptibility under greenhouse conditions and natural field infection. Griffiths (5) concluded that all strains of corn were susceptible to smut at any age if sporidial suspensions were injected into very young growing tissue.

Kühn (8), Brefeld (2), Arthur and Stuart (1), and Piemeisel (9) noted that corn seedlings once infected with *U. zae* were usually killed by the organism. However, Brefeld, Piemeisel, and a number of other workers have concluded that seedlings are less susceptible than plants beyond the seedling stage.

It is obvious that there are rather conflicting reports on the effect of climatic factors upon infection and the prevalence of corn smut, and on the relative susceptibility of seedlings to infection. Therefore the investigations reported herein were undertaken.

METHODS AND MATERIAL

In studying the effect of certain environmental factors on the prevalence of smut, use was made of the percentages of smut-infected plants in selfed lines of corn grown in an artificially induced smut epidemic from 1922 to 1927, inclusive. The methods of planting the seed, producing the smut epidemic and taking notes have been described in detail in previous publications (6, 7). The reports of the United States Weather Bureau Office at St. Paul, Minnesota, were used as the source of information relative to the temperature, amount of sunshine in per cent of possible, and number of days on which precipitation occurred during the corn-growing period.

A study was made also of the correlations between the degree of smut infection of plants grown under smut epidemic conditions in the field with the extent of infection obtained by hypodermic inoculation of plants grown both in the field and greenhouse from seed of the same strains of corn. Seed of 34 lines from a recombination of 8 low smut lines was planted in the "smut plot" and the plants grown under artificially induced smut epidemic conditions. Twenty plants of each line were also grown in another field. When the plants were about 3 feet high, the growing point of each was inoculated hypodermically with a suspension of sporidia of 8 physiologic forms of *U. zae*. Seedlings of these same 34 lines were grown also in sand in the greenhouse and inoculated hypodermically when about 6 inches high with the same 8 physiologic forms. The forms of smut used were M2 to M9, inclusive, all of which had been isolated from smut galls collected at University Farm.

In order to eliminate errors due to chance infection, notes were taken only on the number of galls formed at the point of inoculation for plants hypodermically inoculated in the field. The seed of each line grown in the

greenhouse was planted in three uniformly replicated rows on November 9, the seedlings were inoculated on November 30, and notes were taken on December 8. The seedlings were watered once with a nutrient solution. Only seedlings which developed smut galls were classed as infected. The temperature from date of inoculation until the notes were taken ranged from 75° to 80° F.

EXPERIMENTAL RESULTS

Graphs are presented in figure 1 for mean temperature, sunshine in per cent of possible, and number of days on which more than 0.01 inches of precipitation occurred during the months of July, August, and September. The data are summarized for each two weeks period. The graphs are constructed from averages for the first 15 days and the remainder of each

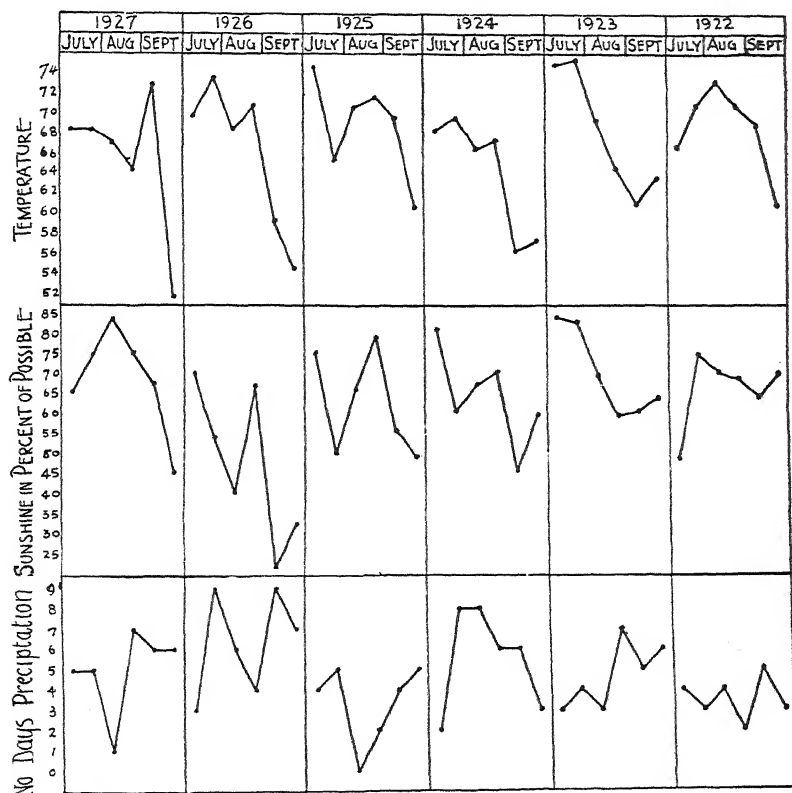


FIG. 1. Graphs showing the average temperature, sunshine in per cent of possible, and number of days on which over 0.01 inches of precipitation occurred, by two-week periods, during July, August, and September, 1922-1927.

month, as determined from the monthly weather reports of the United States Weather Bureau Office at St Paul, Minn.

In table 1 are presented the smut reactions of the same selfed lines of corn which were grown in an artificially induced smut epidemic for two or more years. Notes were taken at three periods of development in 1922 to 1926 (inclusive) and twice in 1926 and 1927. The percentage infection on any particular date was the total amount of infection at that time. For example, midseason infection included early infection as well as infection occurring after the first note was taken. The final note on total smut infection included all infections on the selfed lines. Because of its economic importance, ear smut was calculated separately.

TABLE 1.—*Percentage of total and ear smut infection on selfed lines of corn (selfed 5 years or more) grown in an artificially induced smut epidemic when notes were taken at different periods*

Number selfed lines compared	Early infection							Mid-season infection						
			July	Aug.	July	July		Aug.	Aug.	Aug.	Aug.	Aug.	Aug.	
			22	1	20	19		23	17	18	26	21	14	
	1927	1926	1925	1924	1923	1922		1927	1926	1925	1924	1923	1922	
6	—	—	15.3	11.9	11.0	7.3		42.2	17.2	49.7	34.9	51.9	43.4	
74	—	—	—	—	—	—		32.4	17.4	—	—	—	—	
15	—	—	10.5	6.9	—	—		—	23.1	35.8	26.9	—	—	
30	—	—	—	—	—	—		—	19.8	38.1	—	—	—	
23	—	—	11.2	8.4	—	—		—	—	38.1	28.1	—	—	

Number selfed lines compared	Total infection							Ear infection						
	Oct.	Sept.	Sept.	Sept.	Sept.	Sept.		Oct.	Sept.	Sept.	Sept.	Sept.	Sept.	
	3	14	10	25	12	8		3	14	10	25	12	8	
	1927	1926	1925	1924	1923	1922		1927	1926	1925	1924	1923	1922	
6	58.7	46.5	58.9	47.7	56.5	49.4		19.0	16.4	20.5	17.0	15.4	11.0	
74	43.9	33.7	—	—	—	—		8.1	7.8	—	—	—	—	
15	—	42.2	51.8	28.8	—	—		—	7.7	17.7	7.5	—	—	
30	—	40.6	50.6	—	—	—		—	10.0	18.5	—	—	—	
23	—	—	51.4	32.7	—	—		—	—	17.0	9.4	—	—	

The selfed lines of corn used in this study were grown on the same plot of ground every year of the test. It is possible that conditions for infection were more nearly optimum from 1924 to 1927 (inclusive) than during the first two years of the trial owing to the accumulation of smut in the plot as time went on, although the data obtained do not seem to support this possibility.

It seems, from a study of early, midseason, and total smut infection in table 1 and figure 1 that low precipitation and a high percentage of sunshine, *i.e.*, dry weather conditions, were conducive to a high percentage of smut infected plants. The number of days of precipitation in 1927 and 1925 was low, and the percentage of sunshine was relatively high. A high percentage of smut infection resulted. In 1926 more rainy days were encountered with a lower percentage of sunshine. A lower percentage of smut infection was obtained. In August and early September of 1924, the number of days of precipitation was fairly high with percentage of sunshine slightly lower than in 1925 or 1927, resulting in a lower percentage of smut infected plants.

The correlation between the number of days of precipitation and percentage of ear smut infection, which occurs primarily from August 15 to September 15, is clearly indicated. During the years 1927 and 1925, when the percentage of ear smut was highest, the number of days of precipitation was low and the percentage of sunshine was high. In the years 1926, 1924, and 1923, in which the percentage of ear smut was lower, the reverse conditions were, in general, true.

A significant correlation between temperature and smut infection was not apparent. Temperature did not seem to be a limiting factor in determining the prevalence of smut under the conditions of the experiment. The number of days of precipitation and the amount of sunshine in per cent of possible were of much greater importance than temperature in determining the prevalence of smut in any particular year, in an induced smut epidemic.

Very few data are available regarding infection of corn seedlings in the field with *U. zae*. Such seedling infection seems to be of comparatively rare occurrence, but when it occurs the seedlings are frequently killed by the smut organism. There is a possibility that the weather conditions earlier in the season may account for this lack of seedling infection. It has been shown that dry weather favors infection of older plants. It is possible that seedlings do not become infected because the weather during May and June is usually not very dry. On the other hand, it is also possible that the inoculum does not accumulate until rather late in the season. Seedling plants may also be less susceptible to natural infection than are older plants.

Observations for a period of years over the state of Minnesota indicate that corn seedlings rarely become infected with *U. zae*, or at least seldom develop galls. Similar results were obtained, as a result of extensive investigations over a period of five years at University Farm, with corn seedlings grown on smut infected soil. The lack of infection was not due to a scarcity of inoculum, since the soil in which the seedlings were grown

had been in corn continuously for many years. Furthermore, manure inoculated with chlamydo-spores was applied to the soil each year while the corn was yet in the seedling stage.

In order to obtain information on the relative susceptibility of corn seedlings to *U. zeae*, from 20 to 40 seedlings about 4 to 6 inches high of 34 F_2 recombination lines were hypodermically inoculated with a mixture of sporidial cultures of eight physiologic forms of *U. zeae*. These seedlings were grown in sand in the greenhouse benches. Nearly all of them became infected. Most of the seedlings were killed by the smut. None of the lines was resistant. On July 8, 1927, in a similar manner, 208 seedlings of normal Golden Bantam sweet corn were inoculated in the field, and 182 of them became infected. Of the infected plants, 122 were killed in the seedling stage. On the same day, 234 seedlings, as controls, were injected with a solution in which there were no sporidia. Only one of the controls became infected. About 200 stalks, 2 to 3 feet high, of the same variety, were injected in the same manner as the above controls. Many of these became infected, although this plot was separated from the seedlings by only a few rods. This indicates that inoculum was being blown about. From these results and observations, it is evident that corn seedlings are very susceptible when the inoculum is artificially injected into meristematic tissue, and that they usually escape infection in the field. Weather conditions during the seedling stage of the corn plant were compared with weather conditions during the later stages of growth for the purpose of learning whether the lack of seedling infection was a result of these conditions.

In table 2 is given the prevailing weather conditions, during the growing seasons from 1923 to 1927 at St. Paul, Minn.

TABLE 2.—*The prevailing weather conditions during the growing season of corn from 1923 to 1927, at St. Paul, Minnesota*

Year	Temperatures (degrees F.)				Precipitation (in inches)				Sunshine (in per cent of possible)			
	June	July	Aug.	Sept.	June	July	Aug.	Sept.	June	July	Aug.	Sept.
1923	70.0	75.2	66.9	62.4	4.28	2.51	1.92	1.10	77	84	65	62
1924	63.6	69.0	67.0	56.7	7.24	1.73	6.51	3.05	63	72	70	53
1925	66.2	70.4	71.3	65.3	5.77	4.28	0.16	3.49	64	63	74	53
1926	63.7	71.8	69.7	57.0	3.65	2.92	4.27	5.43	69	62	66	27
1927	63.6	68.3	65.8	62.4	3.98	2.11	1.95	4.27	67	71	79	58
Av.	65.4	70.9	68.1	60.7	4.98	2.71	2.96	3.57	68	70	71	50

There was considerable fluctuation in temperature, in the amount of precipitation, and in the percentage of sunshine during the month of June.

But in none of the five years recorded was there any appreciable amount of smut on the seedlings, although severe infections developed later in the season. The sunshine in per cent of possible was about the same on the average for June, July and August. The average temperature for June was about five Fahrenheit degrees less than for July, and three degrees less than for August. The precipitation was greater in June than in the two succeeding months. On the average it was decidedly greater than that for either July or August. It seems possible that the excessive rains and the relatively low temperatures in June may act as inhibitory factors in smut development. These two factors, however, do not seem to be the chief factors concerned in the scarcity of seedling infection. In 1926, and in 1927, although considerable corn was planted in the latter part of June or in the early part of July and some was planted in August, yet practically none of the seedlings became infected. Thus it seems that corn seedlings in Minnesota usually escape natural infection in the field. The specific reason for this is not known.

In order to test the comparative resistance of more mature plants under natural infection conditions and when hypodermically inoculated, the same 34 F_2 recombination lines mentioned before were grown in the field. Seedlings of the same 34 lines grown in the greenhouse were found to be extremely susceptible when hypodermically inoculated. Plants of these 34 lines, when about 3 feet high, were injected with a conidial suspension of a mixture of eight forms of smut. The results are presented in table 3.

The reactions of these 34 lines of corn when about 3 feet high were quite different from the reaction of the same lines in the seedling stage to the same forms of smut when the plants were hypodermically inoculated. A large number of the lines were highly susceptible when artificially inoculated when about 3 feet high. Some, however, were intermediate in reaction; and a few seemed quite resistant, even when the smut organism was injected directly into the plants.

The correlation between natural infection in a smut epidemic and artificial infection at the location of hypodermic inoculation, of plants of the same lines grown in separate plots, was $+0.40 \pm 0.10$ (Table 3.) This would suggest that there was a certain amount of physiological resistance in these lines to attacks by the smut organism. This type of resistance has been demonstrated by Christensen and Stakman (3). Lines 69, 70, and 75 showed a high degree of resistance to infection both under natural infection conditions in a smut epidemic and when hypodermically inoculated with a mixture of 8 physiologic forms of smut. Lines 48, 68, and 77 were fairly resistant to natural infection but were quite susceptible when hypodermically inoculated. Resistance of these lines to smut infection under normal field conditions may have been largely morphological.

TABLE 3.—*Percentage of smut infection in 34 F₂ lines of corn when naturally inoculated and when artificially inoculated with a suspension of sporidia of 8 physiologic forms of Ustilago zeae*

1927 culture no.	Percentage infected plants		1927 culture no.	Percentage infected plants	
	Natural infection	Hypodermically inoculated in field		Natural infection	Hypodermically inoculated in field
44	25.6	60.0	62	25.0	10.0
45	27.8	45.0	63	26.7	15.0
47	41.3	90.0	64	35.5	0.0
48	2.8	55.0	65	41.9	15.0
49	24.3	85.0	66	33.3	10.0
50	42.6	85.0	67	20.0	10.0
51	32.1	60.0	68	12.5	35.0
52	26.8	65.0	69	18.0	5.0
53	28.8	60.0	70	23.4	5.0
54	55.0	90.0	71	56.9	75.0
55	38.6	100.0	72	30.4	50.0
56	25.0	65.0	73	54.3	85.0
57	27.0	15.0	74	34.0	70.0
58	30.9	5.0	75	18.4	5.0
59	26.9	75.0	76	17.6	35.0
60	30.2	15.0	77	15.7	50.0
61	37.5	25.0	78	28.9	20.0

DISCUSSION AND CONCLUSIONS

From a study of the reactions of selfed lines grown several years on the same plot in a smut epidemic, it could be concluded that low precipitation, high percentage of sunshine, and moderately high temperatures, in other words, dry weather conditions, were most conducive to the production of a high percentage of smutted plants. Temperature was found to be of somewhat less importance in determining the prevalence of smut infection than either number of days of precipitation or sunshine in per cent of possible.

Observations over a period of years indicate that corn seedlings growing in the field rarely become infected with smut, even when the organism is present in abundance. When artificially inoculated in either the greenhouse or field, corn seedlings have proved to be very susceptible to infection. Under normal field conditions in Minnesota these seedlings usually escape infection. The specific reason why corn seedlings escape infection in the field is not definitely known. It seems reasonable to suppose, however, that seedlings must be morphologically or functionally resistant to natural infection in the field. Such resistance is often found among selfed lines of mature corn plants. The location of smut galls on the plants of a

strain is often a strain characteristic. Some lines are highly susceptible to infection at one location on the plants and highly resistant to infections on other parts of the same plants.

F₂ lines from a recombination of 8 selfed lines were grown in a smut epidemic. The same lines were grown in another plot and 20 plants of each line hypodermically inoculated with a mixture of 8 physiologic forms of smut sporidia when the plants were about 3 feet high. The correlation between the percentage of smutted plants in the same lines in these two tests was $+0.40 \pm 0.10$. Certain strains were resistant under field conditions as well as when artificially inoculated. Others were fairly resistant under normal field conditions but quite susceptible when hypodermically inoculated. All of these lines proved to be thoroughly susceptible when artificially inoculated with the same 8 forms in the greenhouse in the seedling stage.

SUMMARY

1. In artificially induced smut epidemics, the effect of environmental factors on the prevalence of infection in selfed lines and crosses of corn was studied. A comparison was also made of the reactions of lines of corn to normal field infection as well as to hypodermic inoculation with the smut-producing organism in the field and in the greenhouse.

2. Dry weather conditions, as expressed by a low number of days of precipitation and a high percentage of sunshine, were found to be conducive to the prevalence of smut. Temperature was not so important a factor as either number of days of precipitation or percentage of sunshine.

3. Corn seedlings proved to be very susceptible when hypodermically inoculated. Infected seedlings are frequently killed by the smut pathogene. Yet observations and experiments over a period of years indicate that seedling infections with gall formation in the field are of comparatively rare occurrence. The specific reason for this is not known. Environmental factors do not satisfactorily explain this lack of infection. It seems possible that corn seedlings are morphologically or functionally resistant to natural field infection.

4. A correlation of $+0.40 \pm 0.10$ was found between natural infection in a smut epidemic and artificial infection of the same strains of corn by hypodermic inoculation with a mixture of 8 physiologic forms of smut.

5. Some lines of corn were resistant to smut infection under natural conditions as well as when hypodermically inoculated with a mixture of sporidia of 8 forms of smut, when the plants were 3 feet high. Other lines were resistant to natural infection but susceptible when hypodermically inoculated.

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DETERMINATION OF LOSSES DUE TO SMUT INFECTIONS IN SELFED LINES OF CORN^{1, 2}

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The annual loss of corn due to smut, *Ustilago zeae* (Beckm.) Unger, for the entire United States (1922-1925) is estimated at 1.7 per cent of the total crop, or more than 47,000,000 bushels. Under some conditions this loss may be much higher. Losses ranging from 5 to 10 per cent are frequently reported from individual states (7).

Very few data are available on the amount of loss caused by smut infections on individual corn plants. Hitchcock and Norton (4), in 1896, found that in a badly smutted field the infected plants produced one-third less corn than the smut-free plants in the same row. In their study all plants with a trace of smut anywhere on the plant were classed as infected. Clinton (1), in 1900, found that smutted plants produced 8 per cent fewer ears than smut-free plants. Hayes, Stakman, Griffiee, and Christensen (3) studied the effect of ear smut on yield. They found a low negative correlation between the percentage of ear smut and yield index of selfed lines grown in a smut epidemic. The loss in some F_1 varietal crosses grown under normal field conditions varied from 1.4 to 8.8 per cent of the ears. Later, Hayes (2) studied smut reaction and yielding ability of selfed lines which were grown under ordinary field conditions. Very susceptible lines had been discarded before the study was made. There was a low negative correlation between yield and percentage of smutted plants in these selfed lines.

The purpose of the present study was to determine the effect of smut galls of different sizes and in different locations on the plants in reducing yield in selfed lines of corn.

METHODS AND MATERIALS

This study was made on pairs of plants of the same selfed lines of corn growing one foot apart and subjected to the same amount of competition

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² This study was made as a part of the general program of breeding for disease resistance being conducted cooperatively by the sections of Plant Genetics and Plant Pathology.

³ The writers wish to express their appreciation to Drs. H. K. Hayes and E. C. Stakman for helpful suggestions and criticisms while conducting this study.

TABLE 1.—*Extent of reduction in yield of shelled corn from plants of selfed lines of corn (selfed 5 years or more) due to smut galls of different sizes and at different locations on the plants*

Location and size of galls	No. of paired comparisons	Mean yield of plants in grams		Percentage reduction in yield due to smut	Odds that smut reduced yield
		Smut free	Smuted		
First shoot above ground	15	55.03	37.77	31	29.1:1
Second and higher shoots above ground	18	50.50	36.81	27	38.2:1
Total infection below ear	36	52.82	34.14	35	1,266.0:1
Neck infection	20	59.40	27.65	54	>10,000:1
Total infection above ear	35	57.04	28.11	51	>10,000:1
Large infections below ear	14	50.21	23.71	53	4,990.0:1
Medium infections below ear	18	53.81	39.31	27	12.6:1
Small infections below ear	4	57.50	47.38	18	2.5:1
Large infections above ear	15	56.43	3.20	94	>10,000:1
Medium infections above ear	12	50.25	35.54	29	25.3:1
Small infections above ear	8	68.38	63.69	7	3.7:1

from surrounding plants. Each pair consisted of one plant with a single smut infection and another which was free from smut. An effort was made to select pairs of plants in which the only difference seemed to be the fact that one plant was smuted and the other was not. Notes were taken on the size and location of smut galls on the infected plants. The smut galls were classified as small, medium, or large, depending on size. Galls designated as small were less than $1\frac{1}{2}$ inches in diameter. Those designated as large were at least 4 inches in diameter, while those designated as medium were intermediate between small and large. Neck infection referred to galls on the upper node or internode, *i.e.*, just beneath the tassel. Infections of the rudimentary ear buds were classed as shoot infections.

The ears of both plants of each pair were numbered, harvested, and dried to approximately 3 per cent moisture; then the yield of the shelled corn from

each plant was determined. The pairing method was used to determine the reduction in yield due to smut infection by determining the difference in yields between each pair of selected plants. The significance of this reduction in yield was determined by the use of "Student's" tables (5, 6).

EXPERIMENTAL RESULTS

In table 1 are presented the data on the reduction in yield of shelled corn, due to smut infections, on plants of selfed lines of corn. Each smutted plant was compared directly with a non-smutted plant of similar breeding, growing at a distance of one foot.

With the refined methods of analysis used in this study, valid conclusions may be drawn from a comparatively limited amount of material. In table 1 it is clearly shown that reduction in yield of corn is influenced markedly by the size of the smut infections. Infections above the ear reduced yield more than did smut galls below the ear. It might be noted in passing that smut infections below the ear are very largely rudimentary ear bud or shoot infections while infections above the ear are direct nodal or internodal infections. There seems to have been little difference in reduction in yield due to smut galls at the first shoot above the ground and those higher on the stalk.

The reduction in yield (54 per cent) due to smut galls located on the neck of the plants was rather striking. The odds were found to be very great, so that this reduction in yield is not due to chance.

DISCUSSION AND CONCLUSIONS

From the data obtained in the present study it seems likely that the losses caused by corn smut have been underestimated. It is relatively easy to appreciate the losses caused when seedlings are killed, when ears are destroyed, and when stalks are badly distorted. But there has been only meager information regarding the reduction in yield caused by smut galls on otherwise apparently healthy plants. It is evident from the results presented in this paper that the yield of shelled corn is reduced materially when there are smut galls of medium or large size on the plants. It is somewhat surprising that yields are reduced more when the galls are above the ear than when they are below it: 94 per cent as compared with 53 per cent in the case of large galls. There is a positive correlation between size of gall and reduction in yield, which, of course, would be expected.

The methods used in this study make possible an accurate determination of the extent that various types of smut reaction reduce yield. If normal varieties are as seriously injured by smut as selfed lines, and if similar results are obtained in other localities, it will be necessary to modify our ideas regarding the destructiveness of corn smut.

SUMMARY

1. A study was made to determine the effect of smut galls of different sizes and of different locations on the plant in reducing yields.
2. Reduction in yield of shelled corn was influenced markedly by the size of smut galls. The larger the galls on the stalks, the greater was the reduction in yield. Infections above the ear reduced the yield significantly more than did galls below the ear.

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THE REACTIONS OF PLANT STEMS TO FUNGOUS PRODUCTS¹

C. R. HURSH

INTRODUCTION

The studies of Haskell (4), Brandes (3), and Bisby (2) served to arouse an increased interest in the phenomenon of wilting of plants attacked by vascular parasites. These investigations demonstrated that green plant stems wilt when the basal ends are cut and placed in liquids in which parasitic fungi have been cultured. The problem then presented itself as to the cause of this induced wilting and the significance of such experiments in a study of wilt diseases. It was postulated that the pathologic condition in plants infected with certain wilt diseases might be brought about by some fungous products deleterious to the host tissue that had been transported considerable distances through the stem from the point of the fungous attack. Should the presence of such substances actually be demonstrated, it would be an important contribution to an understanding of the physiology of fungous parasitism. A number of papers have appeared in which the subject is considered. This literature has been most recently reviewed by White (8) and Rosen (7).

The conclusions of different investigators regarding wilting phenomena are not all in agreement. The fact that plant tissue is disintegrated by certain fungous filtrates [as first shown by DeBary (1)] has frequently been given only secondary consideration by some investigators in their search for substances particularly active in producing the death of plant cells and at the same time readily transported through the water-conducting system of the plant stem. The methods used have been so different that it is difficult to correlate the results that have been reported. This is decidedly true when the degree and rapidity of wilting of plant stems has been used as the principal test for the injurious fungous products. The writer has had occasion to repeat many of the investigations on the induced wilting of plant stems and to make comparative tests with filtrates and isolated products from cultures of a considerable number of fungi. The present discussion concerns itself with certain methods employed and the validity of the results obtained by these methods. The nature and specificity of fungous products and the methods of culturing fungi for study of their products are not considered here.

¹ This discussion is from a report on investigations of methods of attack for the study of disease resistance in plants, conducted under Rockefeller Foundation for International Education and National Research Council Fellowships.

WILTING OF CUT STEMS

The disintegration of tissue discs has proved to be a useful criterion for the injury that a fungous filtrate may produce upon plant tissues. This method is adaptable to standardization. By contrast to the tissue disc method, the wilting of cut plant stems when placed in fungous filtrates presents many discouraging and questionable features as a test for injurious fungous products.

The effect of age of stem.—The age and condition of the plant stems are factors that greatly influence their wilting response when placed in fungous filtrates. It is a simple procedure to demonstrate that rapidly growing, succulent stems will wilt more quickly than older stems or stems grown under hardening conditions.

Table 1 illustrates the difference in behavior that can be secured by using stems of different ages and conditions in the same fungous filtrate. In this experiment, filtrates from two fungous strains proved to be pathogenic to tomatoes were used, together with a filtrate from *Aspergillus niger*. The cut stems were placed in sterile filtrates from three-weeks-old cultures on a modified Richards' solution. The filtrates were diluted with three volumes of distilled water. A selected variety of Stone tomato was used, although entirely similar results may be secured with other varieties of tomato, and with other plant species.

TABLE 1.—*Relation of age and condition of tomato stem to wilting when placed in fungous filtrate*

Condition of stem	Degree of wilting and leaf injury after 12 hours in filtrate from			
	Sterile culture medium	<i>Fusarium lycopersici</i>	<i>Verticillium albo-atrum</i>	<i>Aspergillus niger</i>
Forced seedling 6 inches high	none	severe	severe	severe
Hardened seedling 6 inches high	do	moderate	moderate	moderate
Normal stem 12 inches high	do	none	none	none
Mature stems	do	do	do	do
Growing tip of mature stem	do	severe	severe	severe

Wilting occurs only in the growing, succulent stems. The leaves on the mature stems do not wilt in 12 hours under the conditions of this experiment, but vascular discoloration toward the cut end of the stem does occur. In the succulent stems this discoloration is less evident or entirely lacking.

From the collapse of the basal cells of the succulent stems it is evident that there are substances present in the fungous filtrate that are injurious

to plant cells. The fact that the leaves of the mature stems do not wilt may indicate that the injurious substances are carried upward through the vascular system and subsequently deposited or otherwise rendered inactive before they reach the cells of the leaf tissue. Associated with this inactivation there occurs the discoloration of the vascular system and an interference with the transpiration stream. A consequent pathogenic condition will then manifest itself by the wilting of the upper plant stems.

From the data presented in table 1 it is obvious that degree and rapidity of wilting are not adequate for determining the injury by fungous filtrates to plant stems. Observations must be made on the nature of the actual injury that results. In some cases there is a rapid collapse of the cells at the cut surface. The transpiration stream is stopped, and the consequent wilting of the upper leaves takes place almost as rapidly as though the cut stem had been allowed to wilt normally without contact with any liquid whatever. If such a stem is placed in fresh water after recutting the basal end it may be expected to recover, as previously pointed out by the writer (6). It has also been shown that succulent stems kept in fresh water or in dilute salt or sugar solutions for 24 hours previous to being placed in the fungous filtrates show a different reaction from stems cut and placed in the fungous filtrate at once.

Rapid and gradual wilting.—The phenomenon of rapid wilting and recovery occurs in succulent stems and at higher temperatures. As the wilting that has attracted the attention of certain investigators has been the rapid wilting of succulent stems, the question as to whether or not the stems will recover is a significant one. It is important in all cases of rapid wilting to remove a number of plants before they become dry and to test their recovery in fresh water after a portion of the stem has been cut away at the basal end to insure a freshly cut surface exposed to the liquid.

Besides rapid wilting, a gradual wilting of plant stems may be brought about in fungous filtrates. A considerable time is required before the stem is completely wilted. This type of wilting is more frequent in woody stems. Yellowing may occur, accompanied by vascular discoloration. Rapid recovery in fresh water has not been observed.

In the two types of wilting contrasted above, both of which can be brought about by the same filtrate, the difference seems to be partly in the nature of the water-conducting cells of succulent and woody stems. In rapid wilting one asks whether or not the filtrate is actually being carried into the stem. The gross wilting phenomenon is therefore not so important as is the effect of the injurious substances on certain localized cells or tissues in the stem.

VARIATIONS IN PLANT REACTIONS

Many different types of wilting and other responses may be described for plant stems placed in fungous filtrates or in any solutions containing substances injurious to plant cells. Some substances are carried through the stem and kill small, irregular patches of leaf tissue with little injury to the rest of the plant. Other substances blacken the leaf veins, either with or without producing wilting of the entire stem (see fig. 1). Many necrotic conditions of plant tissue that may be brought about in the laboratory by products isolated from fungus filtrates do not resemble any

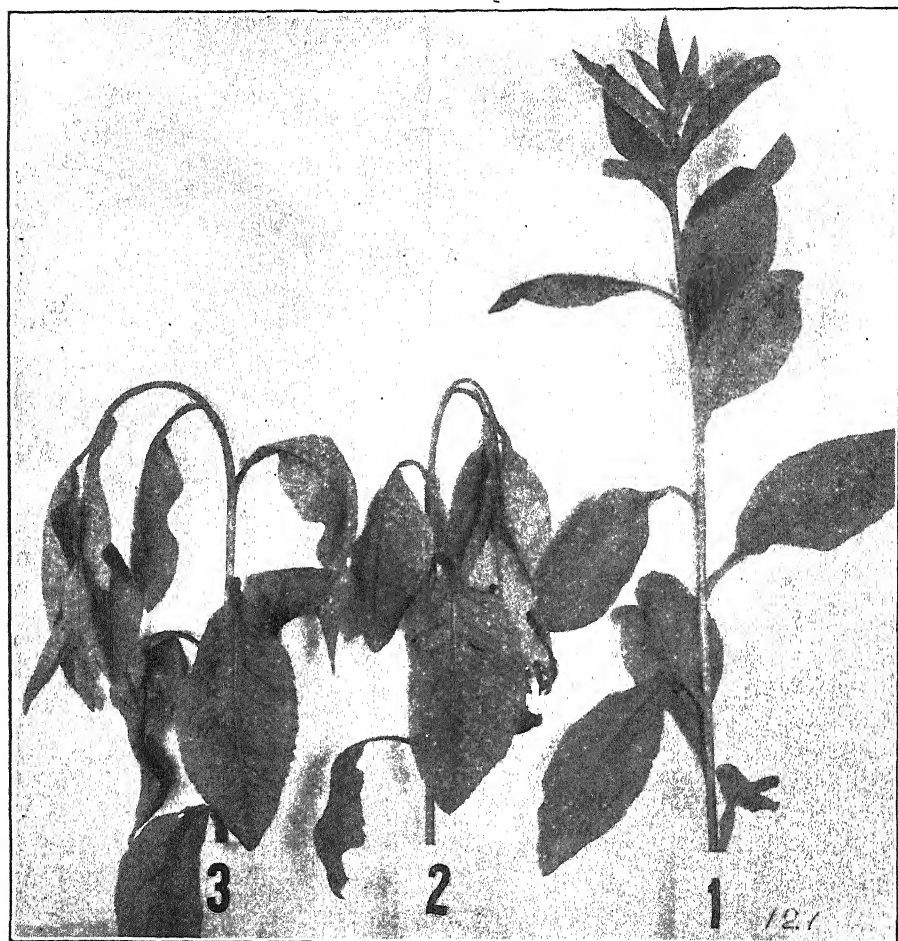


FIG. 1. *Solidago* plants showing injury from products of *Fusarium oxysporum* Schl. This injury is not analogous to pathologic conditions appearing in the field. Note the darkening along the veins, while the remainder of the leaf is still green.

pathologic conditions observed in diseased plants in the field. For example, the necrotic injury to plant stems that can easily be induced in the laboratory by salts such as nitrites may have no direct analogy to pathologic conditions occurring in the plants attacked by wilt-producing fungi. Figure 1 illustrates a typical salt injury that can be secured in the laboratory by products isolated from fungous filtrates where the medium is of the nature of Richards' solution. This type of injury is not recognized as a common pathologic condition brought about by parasitic fungi. This lack of correlation between wilting of stems in the laboratory and in the field emphasizes the importance of studying particular plant tissues that have been affected. For this study, observations with plant stems placed in fungous filtrates are useful in comparing the effect of known and unknown substances upon the vascular system of the plant. The observation should be of vascular discoloration and development of pectic or other hydrophylic substances produced by plant tissues in response to stimulation.

TEMPORARY WILTING

In the well-known wilt diseases produced by species of *Fusarium* and *Verticillium* it is a matter of common observation that, where woody stems are concerned, dry, hot, windy days will produce wilting in attacked plants to a greater degree than in unattacked plants. But even the attacked plants recover their turgescence when the conditions of excessive transpiration are removed. This type of wilting may be different from the final wilting of attacked plants in the field, but it suggests that the difference may be one of degree only.

The fact that attacked plants may recover from the temporary wilting indicates that the condition is due to some interference with the water absorbing and conducting systems of the wilted plants, rather than to any poisoning of the upper leaves by fungous products. When the stem at the point of attack has reached advanced stages of disintegration, the products of the dead cells may themselves be as much responsible as are the fungous products for the pathologic condition of the upper stem. The final wilting and dying occur only after considerable functioning tissue of the plant has been destroyed by the pathogen.

With a few questionable cases excepted, the pathology of tree diseases does not indicate that fungous products are transported very far from the point of attack of the fungus. As a rule, a complete girdling of seriously injured cambium is necessary before the upper limb manifests pathologic conditions.

The common Botrytis wilt of peony is not a vascular disease, but it illustrates a point in the wilting of diseased plants. The roots of diseased

plants are not affected. The wilting is rapid after it once begins, requiring only one or two days for the stem to become dry. There is little or no yellowing prior to the appearance of the wilt. If the individual stems in the clump are observed daily, it will be seen that the *Botrytis* lesions appear at the base of the stem and develop to a considerable extent with no symptoms of injury appearing in the leaves. As soon as the lesion completely encircles the stem the leaves will begin to wilt. On the first day after the symptoms of wilting appear if the stems are cut with a sharp knife a short distance above the lesion and placed in fresh water, the leaves will return to their normal turgid condition and will remain in this state as long as will normal, unattacked stems. Table 2 gives the result of observations.

TABLE 2.—*Recovery of peony stems from wilt after removal from the diseased plant to fresh water at 20° C.*

Condition of stem when cut	Hours elapsed after wilt first observed	Condition of stem after 2 hours in fresh water	Condition of stem after 24 hours in fresh water
Sound	—	turgid	turgid
Flaccid, beginning to wilt	6	do	do
Badly wilted, not drying	18	recovering	do
Drying	30	drying	dead

These observations indicate that wilting has been due to interference with the water conducting system of the plant, and that the upper stem and leaves have not been seriously injured by substances given off by the fungus. It is known that *Botrytis* produces organic acids in culture and that these acids are particularly injurious to plant tissue. However, the wilting of the upper peony stems as observed in the field evidently is not due to organic acids that have been transported through the stem. At the same time, there is good evidence that the killing of plant cells at the point of lesion is directly due to organic acids. Higgins (5) has recently reported this to be the case in the parasitism of *Sclerotium rolfsii* Sacc., and has given an historical review of the subject. A more extensive literature review is that by Zimmerman (9).

DISCUSSION

In attempting to correlate laboratory studies with pathologic conditions occurring in the field, it appears that wilting is not evidence of the movement of highly injurious fungous products through the stem from the point of attack of the pathogen to the upper leaf tissue of the host. Specific fungous products, such as enzymes and acids, appear to be injurious chiefly to the plant cells that are in close proximity to the attacked

tissue. Where the water conducting system is parasitized or is in direct contact with attacked plant tissues, it would be reasonable to expect that certain fungous products might be transported through the transpiration stream to the leaf tissue. If the transported fungous products are highly injurious, it would be expected that a necrotic injury would result in the leaf tissue. But such is not the pathologic condition of the leaf tissue of plants attacked by the common wilt-producing fungi. The symptoms are a general wilting and debility, suggesting starvation and lack of sufficient moisture from the transpiration stream. The abnormal functioning of the vascular system itself may be induced through noxious substances produced either by the pathogen directly or through the disintegration of the host cells. Observations do not seem to support the hypothesis that pathologic conditions such as yellowing and wilting are due to fungous products directly transported to the leaves.

The evidence that wilting in plant stems may not be due to transported fungous products makes it probable that wilting response alone is not the most desirable criterion of the effects of these products upon plant tissues. Further study of fungous products is nevertheless imperative. Aside from the wilting of stems there are a number of available methods for the study of the effects of fungous products on plants. As an example, for soil inhabiting organisms, the effects of the products of these fungi upon root establishment, root hair development, seed germination, etc., are all fertile fields for investigation. It is a simple procedure to demonstrate that a comparatively dilute filtrate from different species of *Fusarium* will inhibit root development. This reaction may be produced by placing mature tomato stems, showing indications of adventitious roots along the internodes, into large test-tubes containing the fungous filtrates sufficiently diluted to produce no wilting of the leaves. The controls are placed in water or in the uninoculated culture media. The roots on the control stems will begin to develop at once, while those on the stems in the fungous filtrate will develop very slowly or not at all. This particular test is adaptable to studies with entire plants. Injection of sterile filtrates into the cavities of hollow stems and leaves has some possibilities for adaptation to experimental studies. Direct observation of individual cells, root hairs, etc., is even more promising of results. Particularly is the significance of vascular discoloration one of the most desirable focal points for study of the pathology of vascular diseases.

SUMMARY

The reaction of plant stems to fungous filtrates indicates an interference with the normal functioning of the water-conducting system.

Wilting is not, in itself, an adequate criterion for the injurious character of fungous products to plant tissues. The age and condition of the stems, the length of time elapsed after cutting, the treatment to which they are subjected during this period, all influence the rapidity and degree of wilting that will occur when stems are placed in fungous filtrates.

A better understanding of the nature of these reactions is dependent upon further study of the particular cells and tissues that are directly influenced by the injurious substances present in fungous filtrates.

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A POWDERY MILDEW PARASITIZING CHINESE CABBAGE

W. H. DAVIS

A powdery mildew was observed on the leaves and floral parts of Chinese cabbage plants (*Brassica pekinensis* Rupr.) which were growing in vegetable gardens at Amherst, Massachusetts. As no description of this disease and organism on Chinese cabbage was found in our most available phytopathological literature, it seemed advisable to solve the following problems. (1) How severe is the disease and what are its symptoms? (2) Does this organism parasitize other crucifers which are cultured in the vicinity and each of which is parasitized by a powdery mildew? (3) What is the genus and species of the organism?

The mildew was first noticed on September 15, 1925. After this date fortnightly observations were made until November when some of the diseased plants were removed from the gardens, transplanted in pots and cultured in the greenhouse for investigation during the following winter (Pl. XIII, B). However, the disease organism reappeared on other Chinese cabbage plants cultured in the same gardens during the autumns of 1926 and 1927. Some of these diseased plants were also potted and cultured in the greenhouse, as was done in 1925.

The disease first appeared on the leaves of Chinese cabbage as small, puckered, wrinkled areas delimited by the veinlets (Pl. XIII, B, 2-4). About one week later, the uppermost surfaces of these areas turned a yellow-orange or bronze color and the leaf appeared as if infected with a virus disease. Finally, the whole leaf turned yellow and many dead areas of a tan color were scattered over its surface. Later, the whole leaf turned a tan color, died, and was easily severed from the stem (Pl. XIII, B, 1). However, a fine white powdery substance, the conidia and mycelium of the organism, could generally be observed on the upper surfaces of the leaves before the yellow puckered areas appeared. The mycelium on the surface of the leaf was persistent and sub-pannose, but the conidia were comparatively few in number (Pl. XIII, B, 1 and 4). The yellowing and dying of small areas on the facies of the leaves were the most prevalent early symptoms of the disease. However, the organism attacked both the upper and the under surfaces of some leaves and generally rendered them non-usable.

In the gardens, the most damage occurred to the flower stalks (bracts) and pods (siliques) which turned brown and died prematurely. In gen-

eral, the damage was very slight to those leaves which had matured before the organism appeared in the autumn. Two cases were observed where it was believed that the mildew killed the plant. In the greenhouse the injury to the plants was more severe than in the garden, where diseased plants were seldom killed.

The inoculations were performed with potted plants cultured in a damp chamber at 15° C. Small portions of the leaves bearing the organism were laid on the surfaces and in contact with healthy ones. Each inoculation was repeated at least three times and some of them twelve times. The following plants were inoculated with viable conidia from Chinese cabbage (Wong bok): common cabbage (*Brassica oleracea* var. *capitata* L.),¹ Danish Ballhead variety; turnip (*Brassica rapa* L.), Burpee's White Egg; radish (*Raphanus sativus* L.), French Breakfast; Chinese cabbage (*Brassica pekinensis* Rupr.), Wong bok; also plantain (*Plantago major* L.), broad leaf plantain—with green leaf bases; cucumber (*Cucumeris sativus* L.), Davis' Perfect; lettuce (*Lactuca sativa* var. *capitata* L.), New York Head; bean (*Phaseolus vulgaris* var. *humili* Alef.), Golden Wax.

The final results of these inoculations showed that conidia from Chinese cabbage infected common cabbage (Pl. XIII, A), turnip, Chinese cabbage, and radish. All other inoculated plants remained healthy except one series in which three inoculated plantains were infected. Two weeks previous to these inoculations in January, six plantains were removed from the field, potted, and cultured in the greenhouse. All the plants being healthy, three were inoculated with conidia from Chinese cabbage and three reserved as checks. The checks remained healthy while two of the inoculated plants showed infection ten days later. Likewise, 12 subsequent inoculations of other plantains were made and all the plants remained healthy; so it was concluded that infection in the first inoculation was due to some contamination and that this mildew on Chinese cabbage seldom, if ever, infects broad leaf plantain.

Within a radius of one-half mile from the gardens, in which these infected Chinese cabbage plants were growing, a powdery mildew was observed on each of several other plants: turnips, beans, cucumbers, and broad leaf plantain. Chinese cabbage plants were inoculated with viable conidia from each of these plants, and conidia from turnips produced the only infection.

Infected plants of Chinese cabbage, common cabbage, and turnip were under constant observation for the purpose of detecting perithecia or a perfect stage of the fungus. What appeared to be immature or abortive perithecia of the Erysiphe type were observed on the midribs and leaf bases

¹ Latin binomials as listed by BAILEY, L. H. Manual of Cultivated Plants, 1924.

of old leaves from Chinese cabbage and common cabbage, but mature perithecia containing asci with ascospores were not detected. Diseased plants of Chinese cabbage, turnip, and common cabbage were also subjected to various conditions of freezing and thawing under controlled laboratory conditions and under outdoor conditions. Furthermore, diseased plants were under observation in the field and in the greenhouse for two years, but no mature perithecia were observed. In this respect the writer's knowledge of the perfect stage of this fungus is similar to Salmon's statement² concerning *Erysiphe ploygoni* D. C. "No one—knows—what other form it takes or how it hibernates through the winter."

Examination of the organism causing the powdery mildew on Chinese cabbage showed that the conidia were borne singly on the conidiophores (Fig. 1, No. 1, 2, 3). Conidia are cylindrical-elliptical, the ends are rounded and they are not constricted (Fig. 2, No. 1-16). Measurements: limits, 12-18 \times 28-52 microns; standard, 14 \times 42 microns (100 measured); conidiophores averaged 9 \times 58 microns and were composed of 2 to 3 cells (Fig. 2, No. 17, 18, 19); hyphae, 6 microns in diameter (Fig. 1, No. 20).

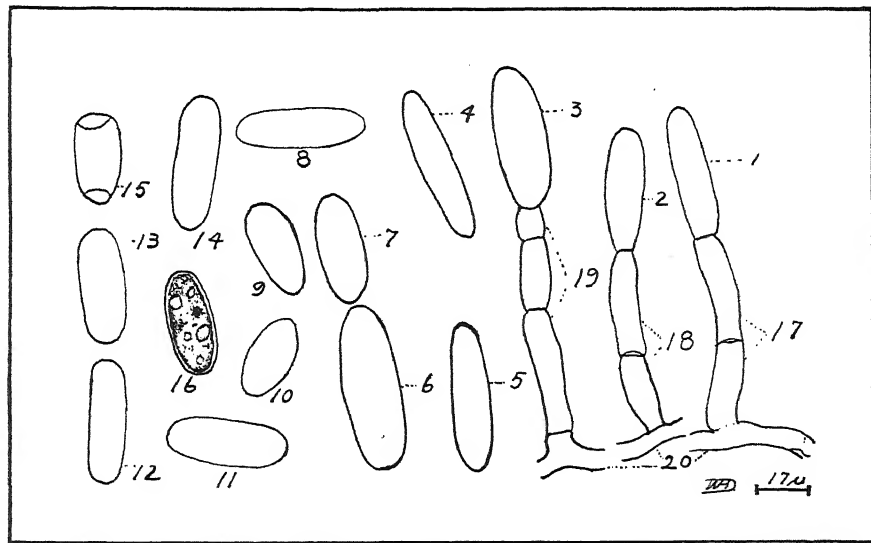


FIG. 1. Conidia and conidiophores of *Erysiphe polygoni* D. C. from leaves of Chinese cabbage. Drawings outlined by aid of a camera lucida. 1-16. Subcylindrical conidia with rounded, unconstricted ends and of various sizes; 1, 2, 3. Conidia borne singly on conidiophores; 15. A non-viable, air-dried, conidium; 16. Thin wall and granular content of a viable conidium; 17, 18. Conidiophores composed of two cells; 19. Of three cells; 20. Hyphae of the fungus.

² SALMON, E. S. A monograph of the Erysiphaceae. Memoirs Torrey Bot. Club 9: 174-191. 1900.

In table 1 are given measurements in microns of 25 viable conidia and conidiophores of mildew removed from the following plants and mounted in water under cover slips: (1) a turnip which had been inoculated with conidia from Chinese cabbage; (2) naturally infected Chinese cabbage plants; (3) a Chinese cabbage plant artificially inoculated with conidia from turnip. Salmon's measurements for the mildew on turnip are included.

TABLE 1.—*Measurements in microns of 25 viable conidia and conidiophores of mildew from four different sources*

Host	Conidium measurements in microns		Conidiophore measurements in microns	
	Limits	Standards	Standards	Cells
Turnip inoculated from Chinese cabbage	11-18 × 30-49	14 × 40	10 × 60	2-3
Turnip (Salmon's mea- surements)	13-15 × 30-40	Not given	Not given	Not given
Chinese cabbage, natur- ally infected	12-18 × 29-47	14 × 41	9 × 58	2-3
Chinese cabbage inocu- lated from turnip	13-18 × 32-49	15 × 40	9 × 60	2-3

Among the above measurements, no decided constant morphological differences were noted in the sizes and shapes of conidia on Chinese cabbage and those on turnip. However, Salmon's measurements indicate smaller conidia—in length—but this is probably due to the fact that he measured conidia from dried, herbarium materials. But, according to Salmon (p. 184), it is inadvisable to consider shapes and sizes of fresh conidia for systematic purposes. Furthermore, other measurements and observations showed that conidia, conidiophores, and hyphae of powdery mildew on Chinese cabbage, turnip, common cabbage, and radish were morphologically identical. In addition to these comparative measurements, inoculations were previously described in which infections were obtained by employing conidia in reciprocal inoculations and inoculations within these four host species. For these reasons, the mildew on Chinese cabbage, turnip, common cabbage, and radish was considered one morphological and physiological species which compares favorably with that reported on turnip by Salmon as *Erysiphe polygoni* D. C. However, physiological forms of this mildew might have parasitized other hosts but this phase of the problem was not investigated.

Finally, the powdery mildew on Chinese cabbage may not be considered a severe disease in this climate. It first appeared in the autumn and caused a yellowing, curling, and dying of the leaves and floral parts. The mildew infected other crucifers, among them turnip, cabbage, and radish, but normal perithecia were not observed on these hosts under the conditions to which they were exposed. The fungus compares favorably with *Erysiphe polygoni* D. C., reported on turnip in Europe by Salmon. It is suggested that this Latin binomial be used for its classification.

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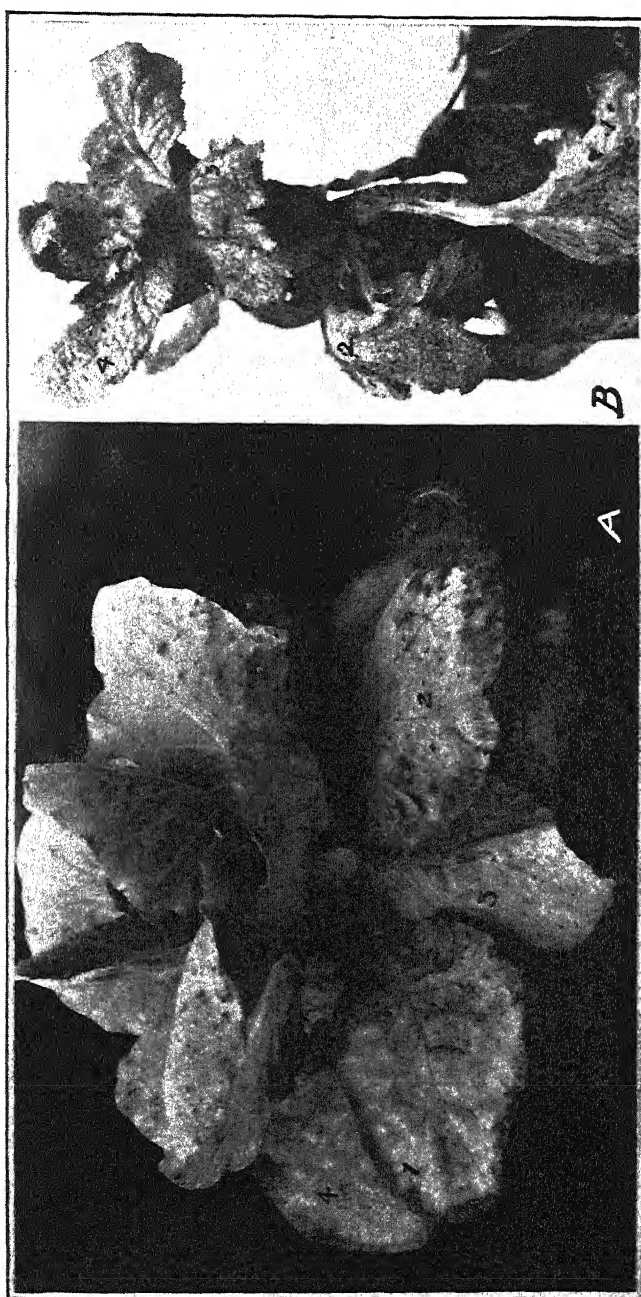
EXPLANATION OF PLATE

PLATE XIII

Photographs of potted plants infected with *Erysiphe polygoni* D. C.

A. An infected common cabbage plant which had been artificially inoculated with conidia from a Chinese cabbage (B). 1, 2, 3, 4. Infected leaves. The mycelium is non-persistent on these leaf margins but pannose on the lower, viable green leaves 1, 2, 4.

B. A naturally infected Chinese cabbage which had been removed from the garden, potted and cultured in the greenhouse for one month. 1. Persistent mycelium on half of a dead leaf; also shown at 4. 2-4. Infected leaves showing symptoms of the disease; puckered areas which have the appearance of mosaic. Many leaf traces show the number of leaves which have apparently been killed by the fungus.



A FIELD METHOD OF INSURING POSITIVE ATTACK WITH SOME CEREAL DISEASES¹

W. W. MACKIE

For many years the writer has been working to breed rust-resistant cereals in California. Stem rust, for instance, under California conditions causes severe injury over the entire state at irregular periods, and in certain localities is the limiting factor in the production of wheat. To test the resistance of hybrid wheat plants in the F_1 , F_2 and later generations, in order to make early separation of resistant hybrid plants or fixed varieties, it was necessary to produce artificially annual attacks of epidemic severity. The method here presented has so successfully met the conditions in California that the writer hopes it may prove useful to breeders of cereals in other regions where conditions are similar.

Greenhouse inoculations for diseases attacking the leaves, it is true, will indicate, in the seedling stage, the mode of inheritance. In the cases of stem rust², leaf rust³, and mildew⁴, several strains or physiologic forms of the organism may be involved in the field attack, while one or more forms have been used in greenhouse inoculations. Identification of physiologic forms of stem rust collected on the University Farm at Davis, California, during a number of years, has shown that the same forms usually occur from year to year. The use of the field attack or epidemic appears workable and practicable for stem rust. It is probably, though not positively, proved that the same conditions would hold for the other cereal disease-producing fungi which possess more than one physiologic form.

Creating an artificial epidemic in the field always has been difficult. It has been observed frequently that wheat plants artificially inoculated with stem rust on the first seedling leaf do not show the same qualities of resistance found later as the plants near maturity.

The writer has observed that many cereal diseases, including leaf rust, stem rust, stripe rust of wheat, and powdery mildew of wheat, oats, and

¹ Investigations conducted cooperatively between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the California Agricutlural Experiment Station.

² STAKMAN, E. C., and M. N. LEVINE. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Minn. Agr. Exp. Sta. Tech. Bul. 8. 1922.

³ MAINS, E. B., and H. S. JACKSON. Physiological specialization in the leaf rust of wheat, *Puccinia triticina*. PHYTOPATH. 16: 89-120. 1926.

⁴ REED, G. M. Varietal resistance and susceptibility of oats to powdery mildew, crown rust and smuts. Mo. Agr. Exp. Sta. Res. Bul. 37. 1920.

barley, halo blight of oats, and barley scald, cause little or no damage until the plants tiller or are in head. Stem rust especially causes its worst damage after the plants are in full head, usually in the milk or soft-dough stage.

In California, the dry season is near at hand when cereal plants are heading. Usually no considerable quantity of rain falls after this time. Increasing heat and drying north winds frequently aid in restricting the progress of cereal diseases of the rust type so that no real indication of the inheritance of resistance can be obtained. A period of two or more such years may occur in sequence. In the meantime the crops of hybrid cereals have been carried through the F_1 and F_2 or even the F_3 generations and beyond the possibility of determining the Mendelian inheritance of disease resistance. The certainty and comparative rapidity which follow the breeding of varieties of wheat resistant to bunt, as compared with the uncertainty and indefinite results of breeding varieties of wheat resistant to stem rust illustrate this point. In the case of bunt, positive infection of a relatively high percentage of the possible number of susceptible plants is secured by the usual methods of artificial inoculation of the seed. No such certainty occurs in the usual field methods employed to induce stem-rust infection.

ARTIFICIALLY INDUCED STEM-RUST ATTACKS

Various devices have been employed to induce stem-rust epidemics in the field. Barberry bushes carrying stem rust have been planted as a hedge surrounding the plats of cereals under test. Sprays containing rust spores have been applied at the critical periods. The writer has applied irrigation water repeatedly at the favorable dates for stem-rust attack. All of these methods, so far as observed, have failed to give a real epidemic, unless the general climatic conditions were favorable, as measured by the stem-rust attack under natural conditions. A satisfactory method of artificially creating an epidemic of stem rust and several other cereal diseases has been discovered and perfected in experiments made by the writer on the University Farm at Davis, California.

FALL DISEASE ATTACKS FOLLOWING JULY SOWING

In breeding for agronomic characters in cereals, it is frequently desirable to hasten the process by growing more than one generation during the year. For years past, the writer has secured a second generation of wheat, barley, and oats in the same year at Davis, California, by sowing seed from the regular June harvest in the July immediately following. Although no rains fall during the summer, these cereals may be germi-

TABLE 1.—*Relation of weather conditions to stem-rust and other cereal-disease epidemics at Davis, California*

	Temperatures in degrees F					Relative humidity in per cent			Rainfall in inches			Cloudiness		Sunshine	
	Mean	Depart- ture from normal	Highest	Lowest	Greatest daily range	5 a. m.	5 p. m.	Mean	Total	Depart- ture from normal	No. rainy days	No. Clear days	No. partly cloudy days	Actual No. of hours	Per cent of possible
March	64.4	+ 6.1	82	43	31	80	49	64	0.36	- 0.62	2	27	1	344	93
April	63.0	+ 4.9	91	46	29	87	58	72	4.25	+ 2.25	7	17	5	282	70
May	64.4	+ 6.1	98	43	31	83	38	60	0.50	- 2.96	1	25	6	399	89
September	67.5	- 1.8	97	47	42	74	30	52	1r	- 0.39	0	29	1	354	95
October	64.7	+ 1.8	87	45	32	76	44	60	2.14	+ 1.10	4	26	3	302	87
November	58.6	+ 5.0	78	41	30	81	59	70	4.48	+ 2.33	11	10	9	144	47

ated, and grown to successful maturity late in November, by means of irrigation. They are then harvested and, after a period of drying, sown for the regular harvest in the next June. At the November harvest many cereal diseases common to the region appeared in epidemic severity.

By sowing both the resistant and susceptible varieties involved in the crosses bred for the genetical study of resistance to cereal diseases, a remarkably certain and positive record of inheritance of disease resistance has been obtained for several successive years.

RELATIONS OF CLIMATIC CONDITIONS TO THE ATTACKS OF STEM RUST AND SOME OTHER CEREAL DISEASES

The spring weather of 1926 over the whole western part of North America, including California, greatly favored the development and spread of stem-rust attacks. A severe epidemic throughout the West Coast States followed, as shown in table 1. The weather data for Sacramento, California, compiled by the United States Weather Bureau, indicates a combination of weather conditions favorable for the development of cereal diseases.

The months of March, April, and May, covering the period in which cereal disease attacks are most pronounced, are compared with September, October, and November, the fall months in which epidemic attacks of cereal diseases were induced by artificial cultural methods. May and September, April and October, and March and November are the comparable months. In temperature, hours of sunshine, and rainfall, the climatic conditions for these paired months closely resemble one another. For humidity and relation of temperatures and humidity to maturity, the changes occur in an inverse ratio for the two periods. In the spring, the temperature increases and the humidity decreases with the advance of the season, but in the fall the temperature decreases and the humidity increases coincidentally with the maturity of the cereals.

The attacks of stem rust and several other diseases are most severe when the plants are nearly mature, or when the kernels begin to form. The increasing temperatures delay maturity at the time when the humidity is increasing. This combination is most favorable to attacks of many cereal diseases. The natural deficiency in humidity in the early fall, as compared with the similar period in the spring, is remedied by the application of irrigation water. This method is invariably effective so far as concerns the creating of favorable conditions for disease attack.

DISEASE EPIDEMICS OF CEREALS INDUCED BY JULY SOWING

The diseases found to be most readily influenced by the method of fall sowing are those which depend upon the air for transfer of spores in natural

infestation. Such diseases must have optimum moisture and temperature conditions. These conditions can be induced at will by means of irrigation and early July sowing. In late October and November, the plants have reached the shooting and heading stages, when they are most susceptible to attacks of wind-borne diseases. At the same time, the optimum conditions for disease development, so far as moisture and temperature are concerned, have arrived. This period of optimum conditions for diseases apparently covers about five weeks after the repressive summer heat has passed and before the exclusively low temperatures of winter have set in.

The effect of the fall weather on maturing cereals is exactly the reverse of that of the spring conditions. All winter-sown varieties tend to mature in a given short period in the spring, but the same varieties sown in July spread the period of their maturity over a much longer time. Some of them fail to mature. By this means the early plants can be separated with ease from the later-maturing ones in families still heterozygous for this maturity factor.

For the past three years, careful and repeated observations on July-sown cereals have shown that the following cereal diseases may be induced in epidemic severity: on wheat, stem rust (*Puccinia graminis*), leaf rust (*Puccinia triticina*), mildew (*Erysiphe graminis*), and spot blotch (*Septoria tritici*); on barley, stem rust (*Puccinia graminis*), leaf rust (*Puccinia anomala*), scald (*Rhynchosporium secalis*), net blotch (*Helminthosporium teres*), spot blotch (*Helminthosporium sativum*), and mildew (*Erysiphe graminis*); and on oats, stem rust (*Puccinia graminis avenae*), crown rust (*Puccinia coronata*), and mildew (*Erysiphe graminis*).

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A POSSIBLE ALTERNATE STAGE OF PUCCINIASTRUM
MYRTILLI (SCHUM.) ARTH.

J. S. BOYCE

In the East it has been shown that the alternate stage of *Pucciniastrum myrtilli* on various Ericaceous hosts is a *Peridermium* on eastern hemlock, *Tsuga canadensis* L. (1, p. 719; 2, pp. 237-238; 3, p. 27; 4, pp. 33-35). In western Oregon and Washington this rust is reported as very abundant on *Vaccinium macrophyllum* (Hook.) Piper, common on *V. ovalifolium* Smith and *V. caespitosum* Michx., and is rarely encountered on other species such as *V. parvifolium* Smith and *Oxycoccus macrocarpus* (Ait.) Pursh. The writer has found it in this region on only the two species first named. *V. macrophyllum* normally grows at the higher elevations in the mountains, being particularly abundant in the Cascades, where it is commonly and intimately associated with mountain hemlock (*T. mertensiana* (Bong.) Sarg.) and to a lesser extent with western hemlock (*T. heterophylla* (Raf.) Sarg.). While more frequently encountered at lower levels, *V. ovalifolium* is sometimes found with *V. macrophyllum*. In spite of the abundance of *P. myrtilli* on these two huckleberries, no *Peridermium* has ever been found on the hemlocks. The only hemlock rust known in the region is *Caeoma dubium* Ludwig, which is confined to western hemlock. The distribution of this hemlock needle rust as observed over a number of seasons does not indicate a possible connection with *P. myrtilli*.

On May 28, 1924, in Clackamas Co., Oregon, at Government Camp, which is located on the south slope of Mt Hood at an elevation of approximately 4,000 feet, a *Peridermium* (F. P. No. 40402, B. No. 1337)¹ of the cylindrical, columnar type was extremely abundant on the 1923 or previous season's needles of Pacific fir [*Abies amabilis* (Loud.) Forbes]. The current season's, or 1924, needles had not appeared, the buds not yet being open. There was no rust found on western hemlock, mountain hemlock, or noble fir (*A. nobilis* Lind.). *Peridermium ornamentale* Arth. was sparingly present on the needles of alpine fir (*A. lasiocarpa* (Hook.) Nutt.). *Peridermium* sp., by which the *Peridermium* on silver fir will be designated in this paper, was so abundant that it seemed there should be some clue to

¹ F.P.=Herbarium of the Office of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture. B.=Herbarium of J. S. Boyce. The collections cited in this paper have been examined by Dr. H. S. Jackson.

the alternate host. This was found in the fact that infected silver firs were invariably close to *V. macrophyllum* and that where the huckleberry did not occur there was no *Peridermium* sp.

Further study in this locality was made on July 18. *Peridermium* sp. on silver fir was abundant, although most of the peridia had ruptured and discharged the aeciospores. In the immediate proximity of the infected firs, *P. myrtilli* in the uredinial stage was abundant on *V. macrophyllum* (F. P. No. 40351, B. No. 1341). Owing to absence from the region during the entire growing season of 1925 no observations were made that year, but on May 2, 1926, *Peridermium* sp. was found to be very scarce, a search of two hours yielding only four infected needles. In 1927 conditions were different. While *Peridermium* sp. was not nearly so generally abundant as in 1924, on June 19 it was quite common locally, being closely restricted to the moister localities along the streams and the fringes of small swamps. On August 21 there was a light infection of *P. myrtilli* on the shade forms only of *V. macrophyllum* and *V. ovalifolium*, always within a few feet of heavily infected silver fir.

It has been found that the age of the needles on which the aecia develop is of diagnostic value in the needle rusts of balsam firs. This *Peridermium* is one of the three in the region which appear on needles one year old. In other words, in 1927 the aecia appear on 1926 needles only. One of these, *Uredinopsis macrosperma* (Cooke) Magn., is found commonly on lowland white fir (*A. grandis* Lind.), and its white aecia, which appear in the spring or early summer, are in decided contrast to the orange-yellow aecia of *Peridermium* sp. *Uredinopsis macrosperma* so far has not been found associated with *Peridermium* sp., but *P. ornamentale* Arth. has been observed each season at Government Camp, confined, however, to alpine fir. While microscopically there is no significant difference between *P. ornamentale* and *Peridermium* sp., the former produces stout orange-yellow aecia, very much flattened laterally. Furthermore, the two species do not accord in their seasonal frequency. In 1924 when *Peridermium* sp. was so generally abundant, *P. ornamentale* was very scarce. On the other hand, in 1926 when *Peridermium* sp. was so exceptionally scarce, *P. ornamentale* was common, while in 1927 when *Peridermium* sp. was locally abundant, *P. ornamentale* was generally so. From the foregoing it seems that *Peridermium* sp. on silver fir is different from any other *Peridermium* in this region appearing on needles of the same age.

Inoculation experiments have so far failed. On May 2, 1926, at Government Camp, seven plants of *V. macrophyllum* were dug from a spot at an elevation of 4,000 feet where there had been considerable infection of *P. myrtilli* in 1924. These were replanted the same day in a garden at Portland, Oregon, at an elevation of 100 feet. Only two of the plants survived

during the season, both remaining free from rust. The only silver firs in Portland are scattered ornamentals, because the species does not grow naturally at such a low altitude. No silver firs are known in the general neighborhood of this garden, and the nearest forest trees probably are 40 miles away. During the winter another plant died. On June 10, 1927, the one survivor was inoculated with *Peridermium* sp., brought from Government Camp, by dusting spores from infected sprays on the thoroughly moistened huckleberry leaves. Again on June 19 the operation was repeated with fresh inoculum from the same locality, and in addition a large spray of heavily infected needles was left resting on top of the huckleberry plant and several smaller sprays were left on the ground at its base. The succeeding days were cloudy with intermittent rainfall. No infection resulted from these inoculations, but this was expected since the huckleberry in the garden at an elevation of 100 feet was at least a month older in development than those growing naturally at 4,000 feet at Government Camp. The leaves of the former were dark green in color, tough and leathery, while those of the latter at the same time were light yellow-green in color, soft and succulent. Then, too, the usual lack of vigor in a plant removed to a new environment where soil conditions, particularly acidity, were quite different from its original habitat very probably influenced its reaction to inoculation.

From the foregoing discussion it seems reasonable to conclude that *Peridermium* sp. on needles of the previous season of silver fir on Mt. Hood differs from other *Peridermia* in this region and that it may be the aecial stage of *Pucciniastrum myrtilli*. This latter possibility is strong enough to warrant inoculation experiments.

This paper is not complete and is based on circumstantial evidence, which in such a complicated group as the needle rusts of balsam firs may be completely misleading, but the information is given with the hope that it may help some future investigator, since the writer can not continue the work.

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OWEN F. BURGER

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GEORGE F. WEBER

Owen Francis Burger, plant pathologist of the Florida Agricultural Experiment Station and State Plant Board since 1920, died January 26, 1928, in the Good Samaritan Hospital at West Palm Beach, from injuries received in an automobile collision Monday evening, January 23.

He was born at Freeland, Pennsylvania, June 8, 1885, the youngest of a large family. At an early age he was left fatherless.

During his boyhood he attended school at Noblesville, Ind., where he graduated from high school in 1904. He entered the University of Indiana in the fall of 1905 and received an A. B. degree from that institution in 1909, having majored in the botanical sciences. Immediately after graduation, he took up his duties as an assistant to the plant pathologist of the Florida Experiment Station. He pursued graduate work in the University of Florida while working as an assistant and received an M.S. from the University in 1911.

While at the Florida Experiment Station, he was assisting Dr. H. S. Fawcett, from whom was undoubtedly received the inspiration and desire to make mycology and plant pathology a life work. At this time in Dr. Burger's career there developed the desire to serve that was so profoundly manifest in later years.

After receiving the M.S. degree he was made assistant plant pathologist of the Florida Experiment Station. He held this position for two years, leaving in the fall of 1913 in order to pursue graduate work at Harvard University. During 1913-14 he was a University scholar at Harvard and during the following two years was a Priscilla Clark Hodges scholar in the same institution. He was granted an M.S. degree from Harvard in 1915 and a D.Sc. in June, 1916.

Dr. Burger was appointed instructor in plant pathology at the Citrus Experiment Station, University of California, in 1916, remaining until 1918, when he was made a pathologist in the United States Department of Agriculture, at Washington, D. C.

While in the Federal Bureau of Plant Industry Dr. Burger had charge of the then recently organized work on market pathology of fruits. For this work his broad experience, wide interests, and ability as a mixer made him particularly well qualified. He was equally at home among the pathologists, the food products inspectors, the wholesalers and fruit brokers, and the freight handlers on the docks. This experience broadened his outlook with respect to the field of plant pathology and thus influenced his development of the department of plant pathology in Florida. In December, 1920, he was made plant pathologist of the Florida Experiment Station in which capacity he was acting at the time of his death.

Dr. Burger readily qualified in that honorable group known as self-made men. His early education was obtained only by means of an unrelenting struggle. He earned his way as he went through college and during his graduate work.

While in college he was popular and was one of the "gang" that was often suspected of indulging in college pranks. His qualities of good fellowship made him equally well liked among the American phytopathologists, where, as every reader of this journal knows, a spirit of comradeship generally prevails. A note from one of his former colleagues includes the following statement: "My own clearest recollections of O. F. and my best memories of him are, I must confess, not associated with our long discussions of the field and possibilities of market pathology, but with certain lively hours at botanical dinners when half a dozen carefully chosen spirits gathered around a table at a discreet distance from the officers' or speakers' table; and Burger was, of course, the center of the fun." He met all comers man to man, and his six-foot-one height and more-than-two-hundred-pounds weight made him an adversary to be considered.

He was married July 22, 1916, to Helen Sanborn Lothrop, of Boston, Massachusetts, who survives him. His home life was most pleasant. He was a loyal, thoughtful, and devoted husband. His wife was his best companion; they lived for each other, she being at his bedside when he passed to the great beyond. As a man Dr. Burger measured up in full. It is to be regretted that one so big-hearted and lovable could not be spared.

Dr. Burger was a devout Christian. In his early life he attended services regularly and was especially interested in young peoples' organizations. He was a member of the Episcopal Church.

Dr. Burger will be remembered by the majority of Florida people for what he did to improve agriculture in this state. His basic education was broad and liberally grounded in the arts and sciences. Practical work in the field and laboratory, conducted parallel with his graduate studies, acquainted him with the problems of the man of the soil. His most important

work was with the citrus grower. He conducted experiments on blue mold decay and stem-end rot of citrus.

A large share of his efforts in the past few years were devoted to improvement in methods of disease control. He took great interest in the functioning of the State Plant Board. He cooperated in formulating and putting into effect state and federal quarantine rules and regulations. He assisted in acquainting railroad employees with the types and amount of disease occurring in transit. Sectional meetings in the state, held for the purpose of conveying recent information from the laboratory directly to the grower, usually found him a principal speaker on the program. His fund of knowledge and his dynamic personality were his outstanding qualities as a teacher and platform speaker. He travelled extensively in the state, visiting farms, groves, and packing houses.

He was alone in the Department of Plant Pathology when he assumed his duties in 1920. It was not long, however, until he was convinced that the need for progress was so great and the problems so numerous that he must have aid. In surrounding himself with assistants he demonstrated his broad vision by selecting men fully prepared to conduct research work. He desired men who were mature, even though inexperienced. He selected them from the larger institutions where leaders in plant pathology were located.

At the time of his death his staff consisted of twelve trained men scattered over the state in the sections where they were most needed, all doing more or less individual research toward a common end, the assistance of the producer and consumer. He demonstrated his leadership and ability as an organizer by developing a large and smoothly functioning department. His ideas of service and his regard for the truth have been instilled into every one of his assistants. His breadth of vision, personality, sense of duty, and ambition were four reasons for his success. His ability as a scientist was equalled, or possibly surpassed, by qualities of leadership and organization which contributed so largely to his success as a director of the numerous activities under his supervision.

The preliminary work of selection of personnel and the organization of projects in plant pathology was almost completed by Dr. Burger. The periods of anxiety and doubt were past. He was beginning to see the end of the formative period and about to enter into the productive field. He was taken from his desk when the load was beginning to lighten. Under ordinary circumstances he would have enjoyed a score of years of productive work in the state with due recognition from the nation.

Dr. Burger was a fellow of the American Association for Advancement of Science and a member of the following: American Phytopathological

Society, Botanical Society of America, American Agricultural History Society, British Mycological Society, Florida Entomological Society, Florida Horticultural Society, New England Botanical Club, Phi Kappa Phi (local treasurer), Sigma Xi (local vice-president), the Masonic Order, and Kiwanis Club.

The following is a chronological list of Dr. Burger's scientific publications:

- A gum-inducing *Diplodia* of peach and orange. *Mycologia* 3: 51-53. 1911 (with H. S. Fawcett).
- A variety of *Cladosporium herbarum* on *Citrus aurantium* in Florida. *Phytopath.* 1: 64-66. 1911 (with H. S. Fawcett).
- Tomato rust. Fla. Agr. Exp. Sta. Press Bul. 207. 1913.
- A bacterial rot of cucumbers. *Phytopath.* 3: 169-170. 1913.
- Cucumber rot. Fla. Agr. Exp. Sta. Bul. 121: 97-109. 1914.
- Cucumber and cantaloupe blight. Fla. Agr. Exp. Sta. Press Bul. 221. 1916.
- Observations on a fungus enemy of the walnut aphid in southern California. *Jour. Econ. Entom.* 2: 278-288. 1918 (with A. F. Swain).
- Sexuality in *Cunninghamella*. *Bot. Gaz.* 68: 134-146. 1919.
- Decay in citrus fruits during transportation. *Cal. State Dept. Agr.* 9: 365-370. 1920.
- Decay of citrus fruits in transit. Fla. Agr. Exp. Sta. Press Bul. 322. 1920.
- Variations in *Colletotrichum gloeosporioides*. *Jour. Agr. Res.* 20: 723-736. 1921.
- Watermelon diseases. *Quart. Bul. Fla. St. Plant Board* 5: 131-138. 1921.
- Peronospora disease of tobacco. *Quart. Bul. Fla. St. Plant Board* 5: 163-167. 1921 (with H. C. Parham).
- Red rot of sugar cane. Fla. Agr. Exp. Sta. Press Bul. 334. 1922.
- Spraying to control melanose. Fla. Agr. Exp. Sta. Press Bul. 335. 1922 (with E. F. DeBusk).
- Spray schedule for peaches. Fla. Agr. Exp. Sta. Press Bul. 336. 1922 (with J. R. Watson).
- Some sweet potato diseases. *Quart. Bul. Fla. St. Plant Board* 6: 71-76. 1922 (with A. C. Brown).
- Green spotting of citrus fruits. Fla. Agr. Exp. Sta. Press Bul. 342. 1922 (with E. F. DeBusk).
- Black rot of oranges. Fla. Agr. Exp. Sta. Press Bul. 343. 1922 (with Wm. Gomme).
- Preliminary report on control of melanose and preparing bordeaux-oil. Fla. Agr. Exp. Sta. Bul. 167: 123-140. 1923 (with E. F. DeBusk and W. R. Briggs).
- Florida citrus diseases. Fla. Agr. Exp. Sta. Bul. (in press) (with A. S. Rhoads and E. F. DeBusk).

Numerous brief and more popular articles have appeared in reports of the plant pathologist of the Florida Agricultural Experiment Station, in Florida Horticultural Society reports, Citrus Industry, Florida Grower, and elsewhere.

THE INHERITANCE OF RESISTANCE TO PUCCINIA GRAMINIS TRITICI IN A CROSS BETWEEN TWO VARIETIES OF TRITICUM VULGARE¹

C. H. GOULDEN, K. W. NEATBY, AND J. N. WELSH²

Wheat varieties that are susceptible to certain physiologic forms of *Puccinia graminis tritici* in the seedling stage often show very high resistance under field conditions. Hayes *et al.* (5) describe the inheritance of rust resistance in a cross of Marquis×Iumillo with Marquis and show that the Marquis×Iumillo resistance shown in the field is dependent on at least two genetic factors which are inherited apparently independently from a single factor for immunity from certain physiologic forms under greenhouse conditions. In this instance the resistance shown in the field is not necessarily a different type of resistance from that shown in the greenhouse. Physiologic forms other than the group to which the Marquis×Iumillo parent is immune were evidently present in the field, and the results indicate that a different set of factors were operating for resistance to these forms. Aamodt (1) points out that the reaction to particular physiologic forms shown by seedling plants in the greenhouse may not necessarily be a criterion of the reaction in the field and in addition gives an excellent review of published data on this phase of rust investigations. Aamodt states that, "Immunity or a high type of resistance can be differentiated in the seedling stage in the greenhouse with the expectation that the strains probably also will be resistant in the field. Moderate resistance, however, must be differentiated under field conditions in order to have an accurate determination of the reaction of the plant." The question as to the value of a greenhouse test for resistance is therefore left somewhat in doubt. In order to answer the question satisfactorily it is evident that tests conducted on plants approaching maturity must be carried out under conditions such that the physiologic forms may be controlled. The lack of agreement between field and greenhouse results may be due on the one hand to additional rust forms that are present in the field and on the other hand to the expression in plants approaching ma-

¹ The writers wish to acknowledge very gratefully the assistance of Dr. Margaret Newton and Messrs. T. Johnson and A. M. Brown in the physiologic form work. Mr. R. F. Peterson, student assistant gave valuable assistance throughout the entire study.

² Senior Cerealists and Cerealists at the Dominion Rust Research Laboratory, Winnipeg, Canada. Contribution from the Cereal Division, Dominion Experimental Farms Branch, Ottawa.

turity of a type of resistance that cannot be detected in the seedling stage. An increasing resistance as the plants of certain strains mature does not necessarily indicate an increase in the physiologic resistance shown in the seedling stage but perhaps merely the superimposing of a mature plant type of resistance which may or may not be physiological.

The present study was undertaken in order to obtain further information on the inheritance of high degrees of both seedling and field resistance, and particularly on the relation between the two types of resistance.

PARENT MATERIAL

The resistant parent in the cross studied is known as H-44-24 and was produced by McFadden (7) by crossing Yaroslav emmer with Marquis wheat. This strain has been almost entirely free from rust during the past three seasons in tests conducted by the Botany Division at all of the experimental stations in Western Canada. Occasionally, small pustules may be found just above the nodes but it is quite certain that damage from rust is negligible. Agronomically, H-44-24 is probably not of much value since it is somewhat short and weak in the straw, has rather persistent glumes, and a slightly brittle rachis. As to the milling quality of the grain not much information is yet available but one test has been made

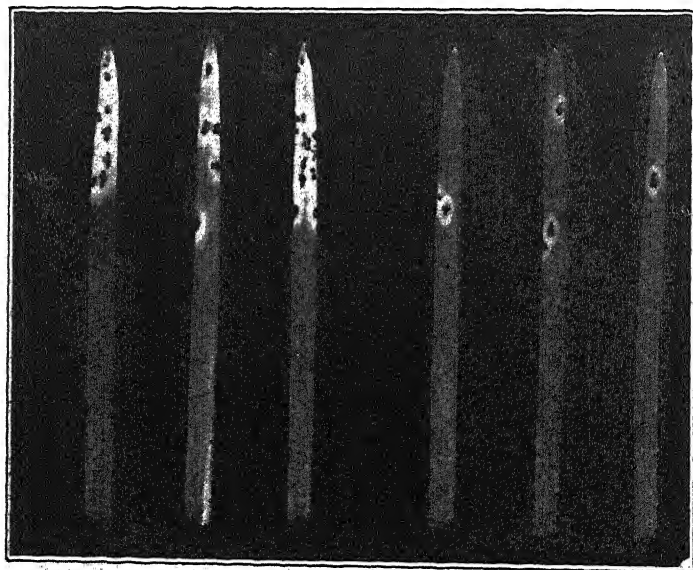


FIG. 1. Seedling reactions of Marquis (left) and H-44-24 (right) to physiologic form 21.

at the Cereal Division Milling and Baking Laboratory at Ottawa. From the standpoint of loaf volume, texture, and color of bread, the wheat was quite satisfactory. The crust was somewhat durum-like in character.

Seedling tests of H-44-24 for rust resistance to seven physiologic forms have been conducted by Newton and Johnson (8), and table 1 is an excerpt from their publication comparing the reactions of H-44-24 and Marquis.

TABLE 1.—*The reaction of H-44-24 and Marquis, common spring wheats, to seven physiologic forms of Puccinia graminis tritici*

Varieties	Host reaction to physiologic forms						
	21	29	30	32	34	36	+ ^a
H-44-24, R.L. 229.....	2 +	2 +	3 -	—	—	0; 1	1
Marquis, R.L. 84.....	4	4 -	4	4 =	4 -	4	2 -

^a An unidentified form.

The reactions of H-44-24 and Marquis to forms 21 and 36 are shown in figures 1 and 2.

Marquis was chosen as the other parent in the cross owing to its desirability, with the exception of rust reaction, for conditions in Western Canada.

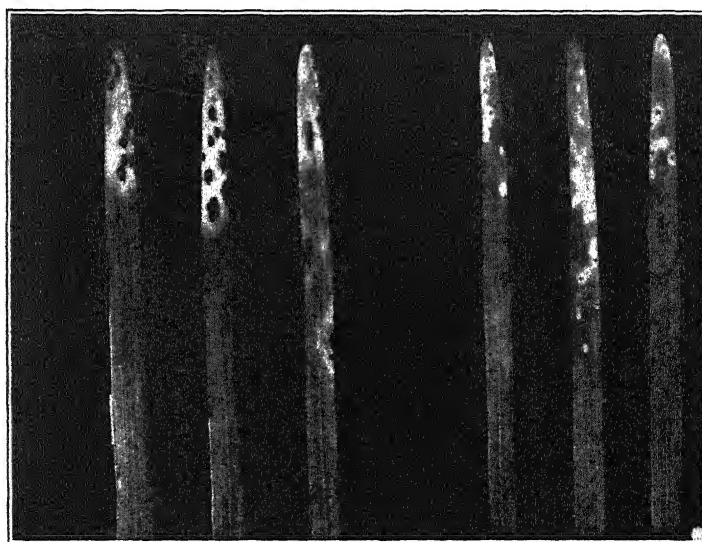


FIG. 2. Seedling reactions of Marquis (left) and H-44-24 (right) to physiologic form 36.

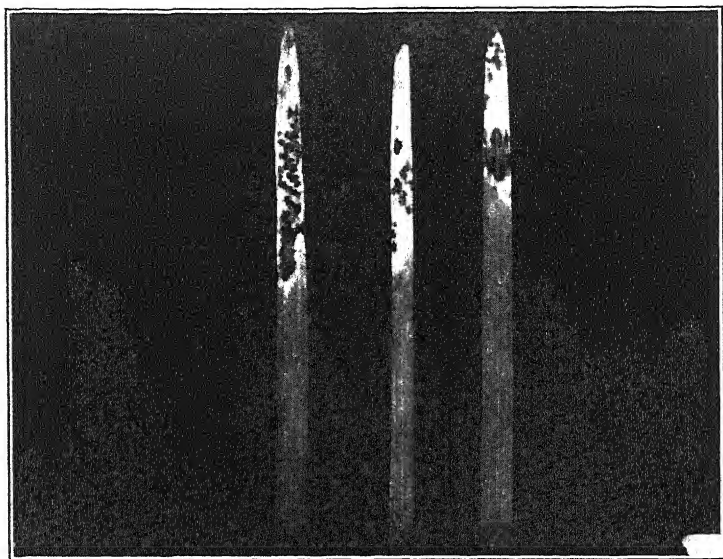


FIG. 3. Seedling reaction of F_1 plants of H-44-24 \times Marquis to physiologic form 21.

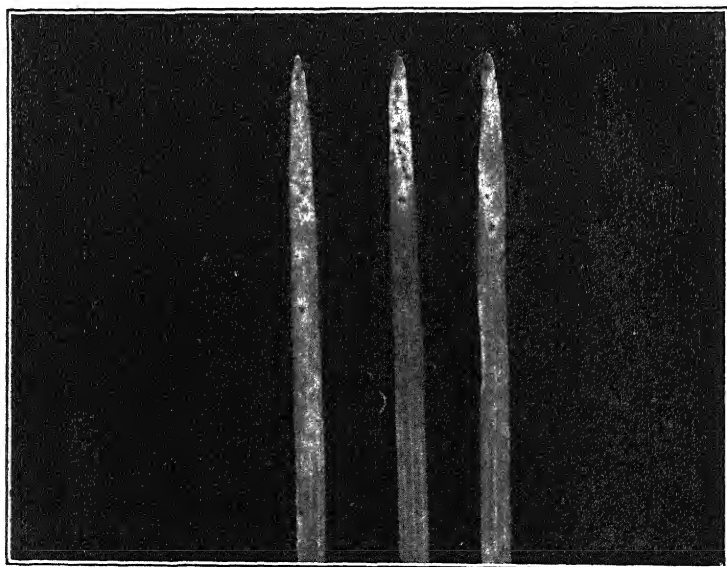


FIG. 4. Seedling reaction of F_1 plants of H-44-24 \times Marquis to physiologic form 36.

THE F_1 GENERATION

The first generation plants were all grown in the greenhouse during the winter of 1925-26. Seedlings of F_1 plants were tested to the two forms 21 and 36 with results as shown in figures 3 and 4.

The moderate resistance of H-44-24 to form 21 is recessive in the F_1 , while the high resistance to form 36 is dominant. As will be shown later this is supported by data on the seedling reactions of the F_3 families.

An interesting feature of the resistance of F_1 plants was observed when these were inoculated with form 21 at different stages of maturity. The results, as given in table 2, and illustrated in figure 5, show that seedling

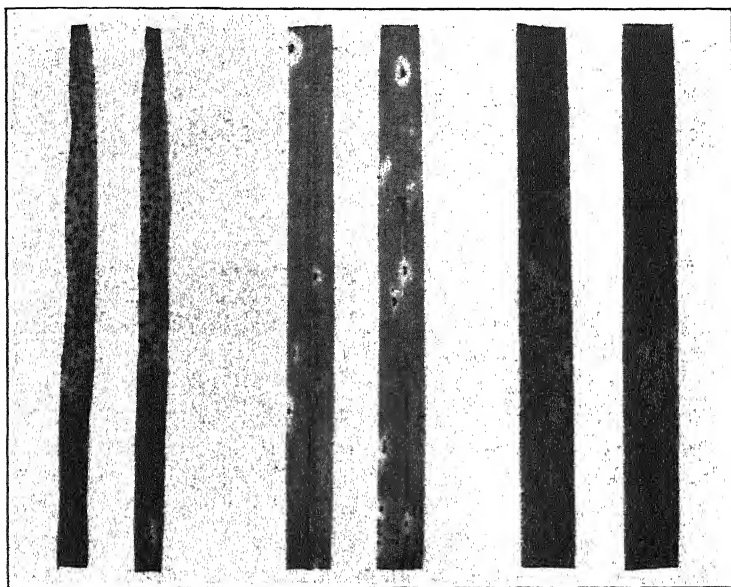


FIG. 5. Leaves from F_1 plants of H-44-24 \times Marquis inoculated with physiologic form 21 at different stages of maturity. Left, seedling stage; centre, shot blade; right, fully headed.

susceptibility in this case changes very quickly to moderate resistance and by the time the plants are fully headed they show almost complete resistance. As evident from the table, each test was conducted in duplicate. This result indicated the possibility of the presence of factors for mature plant resistance which completely covered up the susceptibility evident only in the seedling stage.

TABLE 4.—Numbers of F_3 families of $H-44-34 \times$ Marquis classified by their greenhouse reaction as homozygous susceptible, segregating, and homozygous resistant to physiologic form 36.

F ₁ plant number	Number of F ₃ families			Total no. families
	Homozygous susceptible	Segregating	Homozygous resistant	
550	0	38	12	50
538	2	44	8	54
535	5	50	13	68
534	7	31	9	47
543	13	29	10	52
541	6	36	17	59
537	3	40	4	47
551	4	13	5	22
548	1	23	1	25
533	0	11	1	12
542	1	34	11	46
545	2	50	13	65
546	0	25	11	36
549	1	19	5	25
539	2	7	1	10
540	2	36	10	48
547	0	23	4	27
544	0	39	13	52
Totals	49	548	148	745

It is evident that the ratio of homozygous susceptible families to the remainder is close to 1:15. The actual ratio is 49:696, and the theoretical 1:15 is 46.6:698.4. The deviation 2.4, divided by the probable error 4.46, gives 0.54, showing a very close fit.

The factor relations for resistance to form 36 in this cross are probably as illustrated in diagram 1.

In obtaining the actual data it was often very difficult to distinguish between homozygous R and SR types, so these have been added together in the summarized results as shown in table 5. (It was much easier to distinguish families segregating for R and SR.) The theoretical ratio for the three types is therefore 3 homozygous resistant, 12 segregating, and 1 homozygous susceptible. The comparison between actual and theoretical numbers is also given in table 5.

For three classes P = about 0.6 for $X^2 = 0.81$, Pearson (9), and Fisher (2). The fit is therefore very good.

The F_3 lines tested to form 21 were classified as homozygous resistant, segregating, and homozygous susceptible. By resistance in this case is

DIAGRAM 1. *Probable factor relations in the cross H-44-24 × Marquis for resistance in the seedling stage to form 36*

Parents H-44-24 - $R_1R_1R_2R_2$
 Marquis - $r_1r_1r_2r_2$
 F_1 $R_1r_1R_2r_2$

F_2	Theoretical frequency of genotypes	Genotypes	Phenotypes	Segregation in F_2
	1	RRRR	R	R
	2	RRRr	R	3R + 1SR
	2	RrRR	R	3R + 1SR
	4	RrRr	R	9R + 6SR + 1S
	1	rrRR	SR	SR
	1	RRrr	SR	SR
	2	Rrrr	SR	3SR + 1S
	2	rrRr	SR	3SR + 1S
	1	rrrr	S	S

TABLE 5.—*Numbers of F_2 families, homozygous resistant, segregating, and homozygous susceptible, to form 36, and calculation of goodness of fit to a theoretical 3:12:1 ratio*

Groups	Actual	Theor. (3:12:1)	$(A-T)^2/T$
Hom. R + SR	148	139.7	.49
Segregating	548	558.7	.20
Hom. S	49	46.6	.12
Totals	745	745.	.81 = χ^2

meant the moderate resistance shown by the parent H-44-24 to form 21. The parental types recovered in the F_2 families are similar to those shown in figure 1. Aamodt (1) has referred to the reaction of H-44-24 to form 21 as moderately susceptible. This is perhaps a difference in terminology only. The term resistant was used in our case partly for convenience and partly on account of the hypersensitive areas around the pustules which led Newton and Johnson in 1927 to classify the reaction as 2+. Tests were conducted again in the fall of 1927 using form 21 on H-44-24 and many leaves showed a large number of small pustules without distinct hypersensitive areas. However, during the winter of 1926-27, when the F_2 families were tested, H-44-24 was used as a check and the reaction was consistently that shown in figure 1.

As might be expected, considerable difficulty was encountered in classifying the F_2 families but an attempt was made to make as careful a de-

nination as possible of the families breeding true for the reactions ideal of the parents. The results are as shown in table 6.

TABLE 6.—Numbers of F_2 families of *H-44-24* \times *Marquis* classified by their greenhouse reactions as homozygous susceptible, segregating, and homozygous resistant, to form 21

F_1 plant no.	Number of F_2 families			Total no. families
	Homozygous susceptible	Segregating	Homozygous resistant	
550	7	9	4	20
535	6	9	4	19
534	10	26	5	41
543	12	19	5	36
541	31	16	7	54
537	9	33	8	50
551	30	39	5	74
548	14	38	4	56
533	16	27	2	45
542	16	26	9	51
545	14	40	8	62
546	23	27	1	51
549	14	6	2	22
539	17	26	7	50
540	18	33	2	53
547	10	24	4	38
544	17	42	2	61
Totals	264	440	79	783

Assuming susceptibility to the dominant as indicated by the F_1 reaction, the ratio obtained from the totals in table 6 does not give a good fit either a 3:1 or 15:1 ratio. The goodness of fit is shown in table 7.

Since the fit is closest to a 15:1 ratio, it seems reasonable to assume a inference of two pairs of factors between the parents in this cross for derate resistance to physiologic form 21.

GREENHOUSE TESTS ON F_4 GENERATION FAMILIES

Previous studies by other investigators, Puttick (10), Harrington and modt (3), and Hayes and Aamodt (4), have demonstrated that the resistance of two varieties which react reciprocally to two physiologic forms rust could be combined in a single variety. It is conceivable that in certain cases the factors governing high resistance to one or more forms may govern a reaction to one or more other forms and the latter may be by moderate resistance or perhaps susceptibility. In the present study no indications have been obtained that this is actually the case. Four- n F_4 lines which were known to be breeding true for high resistance to

TABLE 7.—Numbers of F_2 families falling into the two classes, homozygous susceptible and segregating, and homozygous resistant, and calculation of the goodness of fit to 3:1 and 15:1 ratios

Ratio	Actual		Theoretical		Dev.	P.E.	Dev. P.E.
	Susc. and segregating	Resistant	Susc. and segregating	Resistant			
3:1	704	79	587.2	195.8	116.8	8.17	14.3
15:1	704	79	734.1	48.9	30.1	4.57	6.3

form 36 and moderate resistance to form 21 were tested in the seedling stage to seven forms, 9, 14, 15, 17, 21, 34, and 36. These all gave reactions similar to those of the H-44-24 parent. Eight F_4 lines breeding true for susceptibility to forms 21 and 36 were also tested to forms 9, 14, 15, 17, and 34. These all gave reactions similar to the Marquis parent including high resistance to form 14. The results are summarized in table 8.

TABLE 8.—Reaction of twenty-two selected F_2 lines of H-44-24 \times Marquis to seven physiologic forms of *P. graminis tritici*

Field reaction	Strain no.	Physiologic forms						
		9	14	15	17	21	34	36
Susc.	7	2 3 -	2 3	3 + -	2 + 3 -	2 3	2 3	
	204	2	2 3	3 + -	2	2 3 -	2 3	1
	293	2 3 -	2 3	3 +	2 + 3 -	2 3	3 - 3	1
	456	2 + 3 -	2 - 1	3 + -	3 -	2 3	2 3	1
	572	3	2 3	3	2 +	2 3	2 3	1
	587	3	2 3	3 +	2 +	2 3	2 3	1
	885	2 + 3 -	2 3 -	2 + 3	2	2 3	2 3	1
	937	3 +	2 3	3 +	3 -	3 -	3 -	1
Res.	128	2 3 -	2 3	3 +	2 3	2 3	2 3	1 -
	386	2 3 +	2 3	3 +	3	2 3	3 + -	1 -
	725	2 3		3 +	2 3	2 3	2 3	1 -
	880	2 3	2 3	3 +	2 3	2 3	2 3	1
	888		2 3	3 + -	3	2 3	3	1 -
	903	2 3	2 3 +	3 + -	2 3	2 3	2 3	1 -
Res.	99	4	1 -	4	3 + -		3 +	
	148	3 +	1	3 +	3 +		3 +	
	179	3 +	1	4	3 +		3 +	
	208	4	1	4	4		4	
	221		1	4 +	3 +		3 +	
	331	3 +	1	3 + -	3 +		3 +	
	450	3 4 -	1 +	3 +	3		3 +	
	875	4	1	4	4 -		3 +	

In this test only those lines were used which were either homozygous resistant or susceptible to both forms, 21 and 36, so it was impossible to determine whether the factors governing resistance to 21 or those governing resistance to 36 were also responsible or closely linked to factors governing resistance to the other forms. A further test was conducted on a group of twelve miscellaneous strains which were varied in their reactions to both forms. The results of this test are presented in table 9. It will

TABLE 9.—Seedling reactions^a of twelve miscellaneous F_4 lines of H-44-34 × Marquis, to physiologic forms 21, 14, 17, and 36

Strain no.	Physiologic forms			
	21	14	17	36
105	Hom. MR	Seg. MR + R	Seg. S + MR	Seg. MS + R
125	Seg. S + MR	Seg. MR + R	Seg. S + MR	Seg. MS + R
248	Seg. S + MR	Seg. MR + R	Seg. S + MR	Hom. R
268	Hom. S	Hom. R	Seg. S + MR	Hom. R
323		Hom. R	Seg. S + MR	Seg. MS + R
349	Seg. S + MR	Seg. MR + R	Seg. S + MR	Hom. R
374	Hom. S	Hom. R	Hom. S	Seg. MS + R
431	Seg. S + MR	Seg. MR + R	Seg. S + MR	Seg. MS + R
490	Hom. S	Hom. R	Hom. S	Seg. MS + R
503	Seg. S + MR	Seg. MR + R	Hom. MR	Seg. MS + MR
504	Hom. MR	Hom. MR	Hom. MR	Seg. MS + R
859	Seg. S + MR	Seg. MR + R	Seg. S + MR	Hom. R

^a S = (3 + and 4 reactions)

MS = (3 - and 3 reactions)

MR = (2 + and 3 reactions)

R = (1 - and 2 reactions)

be observed that there is a close reciprocal relation between the reactions of all but one of the strains to forms 21 and 14. No such relation exists between the reactions to forms 36 and 14. The data with respect to form 17 are not conclusive. Strains numbers 105 to 349 were read before the rust had developed sufficiently to give accurate results. On the remainder, numbers 374 to 859, the results agree quite well with those obtained for form 21. There seem sufficient data to show that in H-44-24 either the same factors govern the reaction to forms 21, 14, and 17, or if these factors are different they are closely linked. The factors for resistance to form 36 are distinct from those governing resistance to the other group of forms. One of the most interesting observations in this connection is the reciprocal relationship between the inheritance of resistance to forms 21 and 14. The Marquis factor or factors for high resistance to form 14 are allelomorphic

to H-44-24 factors for moderate resistance to form 21. In Marquis also the same factors give complete susceptibility to at least two other forms. The factor relationships in the two parents for rust reaction to forms 9, 14, 15, 17, 21, and 34, may easily be as indicated in diagram 2.

DIAGRAM 2. *Hypothetical factor relationships in H-44-24 and Marquis for resistance to physiologic forms 9, 14, 15, 17, 21, and 34.*



If diagram 2 gives a true indication of the relation, it is impossible in this cross to combine the moderate resistance of H-44-24 to form 21 with the high resistance of Marquis to form 14.

It is evident from these results that the building up of resistance by hybridization must be carried out with extreme care in the matter of selection of parents and testing of the progeny to different physiologic forms. The first essential is a thorough genetic analysis of various parents with respect to the factors controlling resistance to different groups of forms. Not until these data have been obtained can a systematic program be outlined to build up resistance to a large number of forms in one variety.

FIELD REACTION OF F_3 FAMILIES

In the summer of 1927 the hybrid nursery was located at Morden, Manitoba, on the plots of the Dominion Experimental Station. The season was very late. Sowing was not completed until June 15. The natural epidemic was very heavy and all of the physiologic forms being carried in the greenhouse were transferred in pots to the field. The result was a very heavy epidemic, which practically destroyed all of the very susceptible varieties and strains.

The 1054 F_3 lines which had been tested the previous winter to forms 36 and 21 were all grown in the field. The parents were scattered uniformly throughout this nursery and, while Marquis was very heavily rusted and produced only very shrunken grain, H-44-24 was almost entirely free and developed normally with the exception of damage caused by foot rots. It did not appear to be more susceptible to such diseases, however, than other standard varieties.

About September 10 data were taken on the reaction of the F_3 lines to rust. They were classified while standing in the field as homozygous resistant, segregating, and homozygous susceptible. The segregation as

illustrated in figure 7 was so clear cut that this classification was easily made. The uniformly susceptible lines were very obvious. The results of this study are given in table 10.

If field resistance is governed by a single factor pair, the three classes should be obtained in a 1:2:1 ratio. The goodness of fit of the numbers obtained to the theoretical 1:2:1 is given in table 11.

This is not a satisfactory fit, owing to a deficiency in the resistant class which is added to the segregating class. There is a possibility that natural

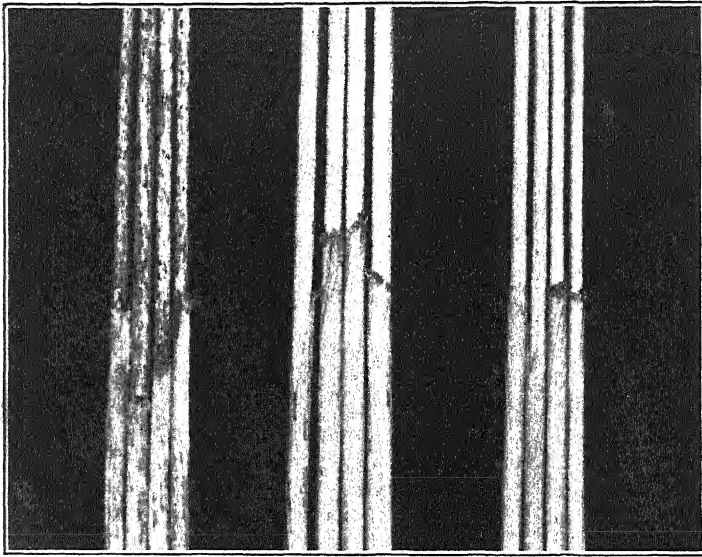


FIG. 7. Field reactions in 1927 hybrid nursery. Marquis, left; H-44-24, right; and a resistant F_2 line, centre.

crossing in the previous generation may have helped to produce such a result. Adding the resistant and segregating classes together, we get 796:249, which is a very good fit to a 3:1 ratio.

RELATION BETWEEN FIELD AND GREENHOUSE RESISTANCE

Any relation between field and greenhouse resistance should be most evident from a study of the families homozygous according to the greenhouse test for resistance or susceptibility to both of the rust forms used. There were 36 of such families susceptible in the seedling stage and 32 resistant. The segregation of these families for rust resistance in the field was studied in detail and the results are as given in table 12. It is evident

TABLE 10.—*Number of F_2 families homozygous resistant, segregating, and homozygous susceptible, as determined by the field reaction*

F ₁ plant number	Number of F ₂ families			Total
	Resistant	Segregating	Susceptible	
550	17	32	9	58
538	20	33	15	68
535	13	35	16	64
534	8	24	15	47
543	9	29	11	49
541	17	27	17	61
537	18	28	14	60
551	17	52	20	89
548	14	46	12	72
533	3	29	15	47
542	15	27	13	55
545	13	43	13	69
549	3	18	8	29
546	10	30	18	58
539	17	27	13	57
547	9	26	8	43
540	7	38	12	57
544	16	26	20	62
Totals	226	570	249	1045

that in both groups there is a 1:2:1 ratio of resistant, segregating, and susceptible families showing complete independence of the greenhouse test. The results are best summarized in the form of a 2-by-3-fold correlation surface, table 13, for which the calculated value of X^2 is 0.275. For $n=2$ (number of degrees of freedom) P is 0.90 to 0.80 indicating complete independence of the two distributions.

Irrespective of this result it cannot be definitely stated that there is not an observable relation between the two types of resistance. There is a

TABLE 11.—*Total number of F_2 families classified for field resistance as resistant, segregating, and susceptible, and calculation of the goodness of fit to a 1:2:1 ratio*

Actual numbers	Theor. numbers	$(A-T)^2/T$
226	261.25	4.756
570	522.50	4.318
249	261.25	.574
1045	1045	9.648 = X^2 P = less than .01, Fisher (2)

distinct difference in the type of segregation in the two groups as shown in table 12. Families in the seedling-susceptible group segregate in the field approximately in a 1:2:1 ratio of resistant, semi-resistant, and sus-

TABLE 12.—*Segregation in the field for rust reaction of two groups of F_3 families*

Homozygous susceptible in seedling stage to forms 21 and 36				Homozygous resistant in seedling stage to forms 21 and 36			
Family no.	Resis- tant	Semi- resis- tant	Suscep- tible	Family no.	Resis- tant	Semi- resis- tant	Suscep- tible
100	All			129	All		
152	do			395	do		
183	do			733	do		
214	do			888	do		
227	do			896	do		
340	do			911	do		
459	do						
883	do						
75	0	40	17	24	27	10	14
256	15	18	10	191	27	1	6
292	2	18	7	278	12	2	10
295	9	23	6	348	14	22	11
419	0	38	17	467	28	6	10
421	3	22	5	560	35	0	15
423	12	41	17	631	47	0	12
470	6	19	8	666	79	2	29
471	20	46	19	670	52	0	20
473	5	23	12	706	29	0	7
615	17	34	13	718	33	0	17
619	10	24	19	719	39	0	9
829	19	35	28	782	64	1	9
836	22	31	18	988	34	8	11
841	14	14	7	192	Segregating nos. small		
854	14	15	13	283			
913	29	29	20	285			
984	4	30	17	502		do	
104			All	57		do	All
155			do	210			do
161			do	302			do
164			do	465			do
233			do	580			do
311			do	595			do
347			do	893			do
621			do	944			do
623			do				
748			do				

ceptible plants, while in the seedling-resistant group semi-resistant plants are almost completely absent. An explanation of this is not possible until a more detailed study has been made of the nature of resistance exhibited only by plants approaching maturity. If such resistance is purely morphological the results obtained from the segregation of these families are probably to be expected. It will be remembered that the SR class contains plants practically free from rust but usually with a few pustules on each plant just above the nodes, and in families susceptible in the seedling stage and segregating for resistance in the field the SR plants would be those heterozygous for the factor for morphological resistance which is not completely dominant. In families resistant in the seedling stage to at least two predominating forms, the chances of the development of rust on only a small portion of the plant surface would be much less, and the SR

TABLE 13.—*Two by three fold correlation tables summarizing results from table 13*

Seedling reaction \ Field reaction	Homozygous resistant	Segregating	Homozygous susceptible	Totals
Resistant to forms 36, 21.....	8	18	10	36
Susceptible to forms 36, 21.....	6	18	8	32
Totals	14	36	18	68

$$X^2 = 0.275. \quad P = .90 \text{ to } .80.$$

class would be accordingly much smaller. In a plant showing only morphological resistance, as described by Hursh (6), there would seem to be greater chances of infection just above the nodes than on any other part of the plant, owing to the fact that the bundles coming out of the node have at that point not extended to the epidermis of the stem.

The exact nature of the resistance here referred to as field resistance is very important. Such families as numbers 100 to 883 in the seedling-susceptible group and numbers 129 to 911 in the seedling-resistant group are indistinguishable from the standpoint of resistance in the field. They are almost entirely free from rust, and if the type of resistance that they show is morphologic they would be expected to show this resistance irrespective of physiologic forms. It is a very pronounced type of resistance and, since it appears to be governed by only a single pair of factors, is extremely important from the plant-breeding standpoint.

GENETIC STUDIES ON OTHER CHARACTERS

Awning

Figure 8 illustrates the spike characters of H-44-24 and Marquis. In F_1 the type was almost intermediate but resembled Marquis more closely than the H-44-24 parent. In F_2 the ratio of awnless and intermediate to awned was 791:267. The deviation from a perfect 3:1 ratio is only 2.5 while the probable error is 9.5. This is a very close fit, and the classification of the F_2 plants was checked by the segregation in F_3 families.



FIG. 8.—Spikes of Marquis (left), H-44-24 (right), and F_1 plant (centre).

Persistence of Glumes

The F_2 plants were threshed with a machine made especially for single plant work. It consists of a drum covered with corrugated rubber which operates against a belt of corrugated rubber extending over about five inches of the circumference of the drum. The belt may be adjusted for tightness against the drum. The heads of a single wheat plant of the ordinary free threshing type are threshed in a few seconds by passing them between the rotating drum and the rubber belt. In threshing the plants in this cross it was observed that there was a fairly clear-cut differentiation between the free threshing Marquis type and the type with persistent glumes resembling H-44-24. The former were threshed by passing them

through the machine once while the others required passing through two to four times. Records were kept, therefore, on the remainder of the plants and a ratio of 540 persistent- to 154 free-threshing plants was obtained. Fitting this to a theoretical 3:1 ratio we have a deviation from the theoretical of 19.5 and a probable error of 7.69. The deviation divided by the probable error is 2.54, indicating that the departure from a 3:1 ratio is not particularly significant.

Pigmentation of Seedlings³

The seedlings grown in the greenhouse for tests of resistance to rust were examined for coloration when about one inch above the ground. A fairly definite segregation was observed and records were kept of the number of families breeding true for color, segregating, and breeding true for absence of color. The ratio obtained was 413:550:54. On the basis of a differentiation between the parents for this character of two pairs of factors the ratio to be expected would be as follows—

7 breed true for color
8 segregate
1 breeds true for absence of color

The goodness of fit of the actual to this theoretical ratio is shown in table 14.

TABLE 14.—*Number of F_2 families breeding true for color, segregating, and breeding true for absence of color, and goodness of fit to a theoretical 7:8:1 ratio*

Groups	Actual	Theor. 7:8:1	$(A-T)^2/T$
Colored	413	444.9	2.29
Segregating	550	508.5	3.39
Green	54	63.6	1.45
Total	1017	1017	$7.13 = \chi^2$ $P = .028$

Since P is less than 0.05 the deviation is quite significant. Table 14 shows that the big discrepancy is in the first two classes, the segregating class being much too large. The only class that was perfectly distinct was the homozygous green class. In the uniform pigmented classes a considerable variation due to environmental influences seemed evident so that a number of these may have been classed as segregating. The ratio of homozygous green families to the remainder is 54:963. The theoretical

³ The coleoptiles of H-44-24 seedlings are of a distinct purplish red color.

1:15 ratio is 63.6:953.4. The fit here is good since the deviation from the theoretical is only 9.6 and the probable error is 5.21. It seems safe to conclude that the pigmentation of the seedlings is governed by two pairs of duplicate factors.

SUMMARY OF FACTOR RELATIONSHIPS

Putting aside for the present the possibility of linkage between any of the factors concerned, table 15 is a fairly accurate statement of the factor relations in the cross studied.

TABLE 15.—*A summary of factor relationships in the cross, H-44-24 × Marquis*

H-44-24		Marquis	
Factor	Character	Factor	Character
R_m	Resistance of mature plants to stem rust	r_m	Susceptibility of mature plants to stem rust
R_1R_2	Resistance to form 36	r_1r_2	Susceptibility to form 36
s_1s_2	do 21	S_1S_2	do 21
a	Presence of full awns	A	Absence of full awns
G	Persistence of glumes	g	Free threshing
P_1P_2	Pigmentation of seedlings	p_1p_2	Green seedlings

$$R_m R_m R_1 R_1 R_2 R_2 s_1 s_1 s_2 s_2 aa GGP_1 P_1 P_2 P_2 \times r_m r_m r_1 r_1 r_2 r_2 S_1 S_1 S_2 S_2 AA ggp_1 p_1 p_2 p_2$$

THE INHERITANCE OF MATURE PLANT RUST RESISTANCE IN RELATION TO

MORPHOLOGICAL AND OTHER CHARACTERS

As previously pointed out, the parent H-44-24 is a vulgare wheat derived from an emmer × Marquis cross. It carries with it in addition to rust resistance other characters derived from the emmer parent. It is bearded, has very persistent glumes, and the coleoptile when just emerging and until the plant is several days old is distinctly of a purplish red color. In these three characters it is sharply contrasted with Marquis. If only a few entire emmer chromosomes were transferred to H-44-24 there would seem to be considerable likelihood of an observable linkage between some of these characters. The segregation for these characters was therefore studied in some detail and an attempt made to detect any tendency for a pair of characters to be inherited together.

The method of measuring such relationships is shown in tables 16 to 30. These are contingency tables in each of which a pair of characters are considered. A direct measure of the relationship between the distribution for the two characters may be obtained by calculating X^2 and determining the value of P from Pearson's tables (9). In determining P from the tables,

Fisher's (2) suggestion has been followed relative to the number of degrees of freedom. Thus in a 3-by-3-fold table there are four degrees of freedom, and one enters Pearson's tables under $n=5$. A summary of the values of P is given in table 31. In interpreting these results it is safe to assume that, if the value of P for any given distribution is higher than 0.05, there is no significant evidence of correlated inheritance. A P of 0.05 means that any association that exists between the two distributions might occur by chance in one out of twenty cases.

TABLE 16.—*Distribution of F_2 families for awning and field rust reaction*

Field rust reaction		Awned	Awning Segregating	Awnless	Totals
	Res.	57	117	51	225
	Seg.	149	291	127	567
	Susc.	57	118	70	245
	Totals	263	526	248	1037

$$\chi^2 = 3.97$$

$$P = 0.411$$

TABLE 17.—*Distribution of F_2 families for awning and seedling reaction to form 36*

Seedling reaction to form 36		Awned	Awning ^a Segregating	Awnless	Totals
	Susc.	13	13	23	49
	Seg.	130	213	203	546
	Res.	35	67	46	148
	Totals	178	293	272	743

$$\chi^2 = 6.24$$

$$P = 0.184$$

^a Data taken on F_2 plants only. The class here referred to as segregating is represented by intermediate-type F_2 plants.

TABLE 18.—*Distribution of F_2 families for awning and seedling reaction to form 21*

Seedling reaction to form 21		Awned	Awning ^a Segregating	Awnless	Totals
	Susc.	67	100	96	263
	Seg.	116	186	138	440
	Res.	18	34	27	79
	Totals	201	320	261	782

$$\chi^2 = 2.49$$

$$P = 0.649$$

^a Data taken on F_2 plants only. The class here referred to as segregating is represented by intermediate-type F_2 plants.

TABLE 19.—*Distribution of F₂ plants for awning and persistence of glumes*

Persistence of glumes		Awned	Awning Intermediate	Awnless	Totals
	Per.	42	60	52	154
	Int.	98	133	112	343
	Free	42	90	65	197
	Totals	182	283	229	694

$$\chi^2 = 4.26$$

$$P = 0.375$$

TABLE 20.—*Distribution of F₂ families for awning and cotyledon color*

Cotyledon color		Awned	Awning ^a Segregating	Awnless	Totals
	Pig.	98	131	138	367
	Seg.	128	195	175	498
	Green	9	26	19	54
	Totals	235	352	332	919

$$\chi^2 = 4.34$$

$$P = 0.366$$

^a Data taken on F₂ plants only. The class referred to here as segregating is represented by intermediate-type F₂ plants.

TABLE 21.—*Distribution of F₂ families for seedling reaction to forms 21 and 36*

Seedling reaction to form 21		Seedling reaction to form 36			Totals
		Susceptible	Segregating	Resistant	
	Susc.	14	113	41	168
	Seg.	15	206	53	274
	Res.	1	40	18	59
	Totals	30	359	112	501

$$\chi^2 = 7.69$$

$$P = 0.106$$

TABLE 22.—*Distribution of F_2 families for persistence of glumes and seedling reaction to form 36*

Seedling reaction to form 36	Persistence of glumes ^a			Totals
	Persistent	Segregating	Free	
Susc.	7	11	9	27
Seg.	57	144	92	293
Res.	15	40	26	81
Totals	79	195	127	401

$$X^2 = 1.01$$

$$P = 0.908$$

^a Data taken on F_2 plants only. The class here referred to as segregating is represented by intermediate-type F_2 plants.

TABLE 23.—*Distribution of F_2 families for cotyledon color and seedling reaction to form 36*

Seedling reaction to form 36	Cotyledon color			Totals
	Pigmented	Segregating	Green	
Susc.	21	27	1	49
Seg.	219	294	35	548
Res.	59	79	10	148
Totals	299	400	46	745

$$X^2 = 1.59$$

$$P = 0.807$$

TABLE 24.—*Distribution of F_2 families for persistence of glumes and cotyledon color*

Cotyledon color	Persistence of glumes ^a			Totals
	Persistent	Segregating	Free	
Pig.	52	122	61	235
Seg.	60	146	90	296
Green	6	14	14	34
Totals	118	282	165	565

$$X^2 = 3.82$$

$$P = 0.433$$

^a Data taken on F_2 plants only. The class referred to here as segregating is represented by intermediate-type F_2 plants.

TABLE 25.—*Distribution of F_2 families for seedling reaction to form 36 and field rust reaction*

Field rust reaction		Seedling reaction to form 36			Totals
		Resistant	Segregating	Susceptible	
	Res.	51	157	12	220
	Seg.	131	395	35	561
	Susc.	58	162	22	242
	Totals	240	714	69	1023

$$X^2 = 3.16$$

$$P = 0.534$$

TABLE 26.—*Distribution of F_2 families for seedling reaction to form 21, and field rust reaction*

Field rust reaction		Seedling reaction to form 21			Totals
		Resistant	Segregating	Susceptible	
	Res.	25	105	64	194
	Seg.	47	284	190	521
	Susc.	21	120	77	218
	Totals	93	509	331	933

$$X^2 = 2.65$$

$$P = 0.620$$

TABLE 27.—*Distribution of F_2 families for persistence of glumes and field rust reaction*

Field rust reaction		Persistence of glumes ^a			Totals
		Resistant	Segregating	Free	
	Res.	35	72	35	142
	Seg.	86	192	112	390
	Susc.	34	81	51	166
	Totals	155	345	198	698

$$X^2 = 1.69$$

$$P = 0.790$$

^a Data on persistence of glumes were taken on F_2 plants. The class here referred to as segregating came from intermediate plants.

TABLE 28.—*Distribution of F_2 families for cotyledon color and field rust reaction*

Field rust reaction		Cotyledon color			Totals
		Pigmented	Segregating	Green	
Res.		87	126	11	224
Seg.		228	310	31	569
Susc.		101	140	7	248
Totals		416	576	49	1041

$$X^2 = 2.82$$

$$P = 0.590$$

TABLE 29.—*Distribution of F_2 families for persistence of glumes and reaction of seedlings to form 21*

Seedling reaction to form 21		Persistence of glumes ^a			Totals
		Persistent	Segregating	Free	
Susc.		49	96	52	197
Seg.		66	188	103	357
Res.		17	20	16	53
Totals		132	304	171	607

$$X^2 = 7.73$$

$$P = 0.104$$

^a Data taken on F_2 plants only. The class referred to here as segregating is represented by intermediate F_2 plants.

TABLE 30.—*Distribution of F_2 families for cotyledon color and seedling reaction to form 21*

Seedling reaction to form 21		Cotyledon color			Totals
		Pigmented	Segregating	Green	
Susc.		105	144	14	263
Seg.		172	248	19	439
Res.		33	43	3	79
Totals		310	435	36	781

$$X^2 = 0.71$$

$$P = 0.936$$

TABLE 31.—*Summary of values of X^2 and P from tables 16 to 30*

Table	Characters ^a	X^2	P	Table	Characters	X^2	P
16	AM	3.97	0.411	25	MR ₁	3.16	0.534
17	AR ₁	6.24	0.184	26	MR ₂	2.65	0.620
18	AR ₂	2.49	0.649	27	MG	1.69	0.790
19	AG	4.26	0.375	28	MP	2.82	0.590
20	AP	4.34	0.366	29	R ₂ G	7.73	0.104
21	R ₁ R ₂	7.69	0.106	30	R ₂ P	0.71	0.936
22	R ₁ G	1.01	0.908				
23	R ₁ P	1.59	0.807				
24	GP	3.82	0.433				

^a Explanations of symbols:

A—awning

G—persistence of glumes

P—Cotyledon color

R₁—seedling resistance to form 36

R₂—seedling resistance to form 21

M—mature plant resistance

Linkages are of course very difficult to detect when one or both of the characters are governed by two pairs of factors, but with regard to the characters represented by A, M, and G the results would appear to be quite conclusive.

DISCUSSION

The existence in such a variety as H-44-24 of a type of rust resistance which does not become evident until the plants are about half way to maturity is of particular significance from the standpoint of breeding work. It appears to be a very pronounced type of resistance amounting almost to immunity and, while there is no definite evidence to the effect that it reacts in the same manner to all physiologic forms, there seems good reason to believe that it does react in the same manner to all of the forms commonly found in the Northwest. From the breeding standpoint, therefore, this entire group of forms may perhaps be considered as an entity. This considerably simplifies the problem especially since we seem to have fairly good evidence that the type of resistance referred to is governed by only a single pair of factors.

Whatever may be the practical value of the results reported here, it is evident that in all breeding problems studies of the inheritance of rust resistance should take into consideration both the seedling reactions and the mature plant reactions of the parents to different physiologic forms. If a mature-plant type of resistance exists in one or both of the parents, it is obvious that a close agreement between seedling and field results is not to be expected.

SUMMARY

1. A cross was made between H-44-24, a vulgare derivative from a Marquis \times emmer cross, and Marquis.

2. The H-44-24 parent is moderately resistant in the seedling stage to several forms of *Puccinia graminis tritici*, including forms 14 and 21, and highly resistant to form 36. Marquis is susceptible to a large number of forms, including forms 36 and 21, but is highly resistant to form 14.

3. The high resistance of H-44-24 to form 36 is dominant in the F_1 generation but its moderate resistance to form 21 is recessive. Tests on 1000 F_3 families clearly indicated that there is a difference between the parents of two pairs of factors for resistance to form 36, and the same is probably true for resistance to form 21.

4. Studies of the reactions of F_4 lines breeding true for reactions to forms 21 and 36 showed that all of the lines possessing moderate resistance to form 21 gave the same reaction as Marquis to this group of other forms. This group of forms contains form 14 to which Marquis is highly resistant and others such as 21 and 17 to which it is highly susceptible. It would seem that in this case the same factors govern resistance to one form and susceptibility to another.

5. First generation plants susceptible to form 21 in the seedling stage showed very high resistance to the same form as they approached maturity. Studies of the field reaction of one group of F_3 lines breeding true for susceptibility in the seedling stage to forms 21 and 36 and of another group breeding true for resistance to forms 21 and 36 showed that segregation in the field was quite independent. The field results indicated that resistance in the field is controlled by a single pair of factors only. \

6. An attempt was made to detect any tendency towards linkage in the inheritance of mature plant resistance, seedling resistance to form 21, seedling resistance to form 36, awning, persistence of the glumes, and cotyledon color. No such tendency was observed.

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PROGRESS OF RUST STUDIES¹

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I count it one of the rare and notable events of my life that I am privileged to meet with fellow scientists in this great northern city. I have always thought of Winnipeg as in the far north, just as I have heard people in New England speak of Indiana as in the far west; and yet I know full well that this is just the edge of a great country of unlimited possibilities that stretches far to the northward, both on the right hand and on the left hand. As I gazed out of the window of the incoming train after crossing the international boundary, I saw no foreign scenery, but just the familiar stretch over rolling prairies that recalled my childhood days in Iowa. So I find Winnipeg in its outward setting very homelike, and no less so in the cordial and sincere greeting I have received. However, it was not only the charm and bustle of a great city and the exhilaration of an expanding territory that induced me to make a thousand-mile trip in winter time in defiance of Boreas, but also the desire to secure first hand information regarding the young, but already famous, Dominion Rust Research Laboratory, the only one of its kind in the world, which is making such notable contributions to the increasingly important problems of the science, and, in addition, to greet my fellow workers whose signatures are familiar but whose faces and voices I do not know. I am grateful for the opportunity you have afforded for this visit, and in return I know you expect me to say something about the rusts.

Of course you and I know what we mean by the term "rusts," but sometimes for the benefit of others we say rust-fungi or plant-rusts and thus screen out all thought of rusts of metals. Yet there are rusts of plants that do not belong in the group of rusts with which we deal, for instance, rust of strawberry and many others. To be still more exact we introduce the term "Uredinales" into titles of articles. This is a comparatively new word, and there is no question what it covers if one happens to know the usage. But it is founded upon the genus *Uredo*, which for a time took in all kinds of fungi, and even today does so to some extent, and is treated among the rusts only as a form-genus. The word *Uredo* comes from the Latin verb *urere*, to burn or blast as with fire or a scorching wind, and is more suitably applied to the smuts than to the rusts.

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In early Grecian times, when Aristotle and Theophrastus wrote, *erysibe* was the term designating the rusts, but it was diverted by Linnaeus and others to the surface mildews. The corresponding word in Latin was *robigo*, which meant just what we mean today, popularly and scientifically, by "rust." But this word also fared badly at the hands of the early scientists. Some years ago I spent considerable effort in ascertaining whether *Robigo* could not be re-established as a good genus of rusts, but all in vain. If I had succeeded, we might have had the pleasing name *Robigales* for the order, a name with a clean historical record. Now when I am asked my special line of study I answer, "The rusts," which I fancy usually conveys about the same exactness of meaning as my reply when I was asked in Germany in *ante bellum* days from what country I came. I proudly answered, "From America—I am an American." But I was humbled by what followed. "Oh, then you probably know some of my relatives in Rio de Janeiro." And I was forced to explain that I had meant only the United States. How could I have said I was a Unitedstatesian?

Before I take up some of the problems of the rusts as they present themselves today, let me outline a few historical episodes to bring to mind the slow unfolding of rust knowledge. It was on the twenty-fifth day of April about the beginning of the Christian era, that the poet Ovid early one morning was strolling along the Claudian way not far out from Rome. This was the time of year when the advent of the wheat rust was especially feared. Down the road came a grave procession of husbandmen and their dependents, headed by a group of white-robed priests. The procession turned into a grove and gathered about an altar; and Ovid, after the manner of writers always alert for material for another article, mingled with the worshippers and recorded the proceedings.

This solemn festival, the Robigalia, which had been held annually for many centuries, was in honor of the god Robigo, who controlled the rust. After the sacred flame was placed on the altar a lengthy prayer was offered, asking the protection of the god that the fields might be free from rust. It was not unlike in phraseology and purport the prayers offered by devout Christians of the present day when drought or some other calamity threatens disaster. After the prayer, in accordance with the customs of the time, sacrifices were offered of wine, grain, and young animals.

What more could have been done? The knowledge of rusts had not advanced materially since prehistoric times. This kind of injury to the crops was popularly supposed to have had its origin in some revengeful act. The best remedies known, aside from prayer and sacrifice, were the sensible advice to avoid wet and low lands, combined with such fantastic methods as sticking sprigs of laurel about the fields and burning piles of straw.

Such ignorance of the nature and treatment of rust changed but little during the next seventeen centuries. In England in 1602, Shakspeare in his play "King Lear" said "a foul fiend" scattered distress among men, beasts, and inanimate objects, including the rust on wheat. A better understanding of the rusts first came when the microscope began to be used during the latter half of the seventeenth century, crude as the instrument was in its early form.

The second episode I wish to present occurred in Florence, a city that encouraged arts and science, greatly antedating the culture of Rome. It was in December, 1729, that a scrupulously dressed man of patrician bearing, well-known as Señor Micheli, passed along the streets with a small quarto in full calf binding under his arm. It contained about two hundred pages and half as many steel engraved plates of plants, mostly of the lower orders, each plate dedicated to a distinguished personage. This volume contained the description and a full page illustration showing gross and microscopic details of the first recognized genus of rusts, that of *Puccinia*, named in honor of Thomas Puccini, a physician in Florence and lecturer on philosophy and anatomy at the Academy.

When you are in Florence go to the Uffizi palace and inspect the statues surrounding the quadrangle. This is the hall of fame for the city of Florence. Near the end of the eastern portico toward the river will be found the statue of Peter Antonio Micheli, the only botanist in this assemblage of eminent personages. It is a dignified, courtly figure, with a sprig of jasmine in the left hand, another sprig together with a boletus and an agaric dropped at his feet, and the right hand supporting the precious quarto of new genera. This volume inaugurated the taxonomic study of the rusts.

As a separate group of fungi, however, the rusts were first recognized about 65 years later when Persoon added the genera *Uredo* and *Aecidium* to that of *Puccinia* with a number of species under each genus. Still later he gave the name Uredineae to the group. In the meantime this orderly taxonomic progress received an upheaval when the younger Hedwig, in an ill-fated study sponsored by A. P. DeCandolle, proposed the genus *Gymnosporangium* to replace Micheli's genus *Puccinia*. The most strenuous and persistent efforts at various times to undo this injury to uredinology have been unsuccessful. The study of the rusts at the beginning of the nineteenth century was now fully launched, and both taxonomy and morphology attracted many able scholars.

The third episode which I have chosen to illustrate the unfolding of the knowledge of rusts is laid in Berlin, and introduces a young man of 22 years of age who had just graduated from the University. It was April,

1853, and from the press had come a treatise of 144 pages on the blight fungi, meaning the smuts and rusts and the diseases caused by them. It would have done credit to a mature mind, but as the work of a boy in his undergraduate days it indicated genius. Besides recording important morphological results, the author traced the course of the mycelium in the tissues of the host and detected the origin of the sori. He argued that, as such fungi were capable of reproduction by spores, they were independent organisms, and not, as most people thought, an excrescence or exudation of morbid tissues. Being independent organisms, they must cause the disease with which they are associated and not be its product, as generally held. Neither did he believe that the teliospores were parasitic upon the uredinia although found in the same sorus.

Thus Anton de Bary settled for all time some of the basic problems of the rusts, although he still maintained the old notions that forms belonging to the genera *Aecidium*, *Uredo*, and *Puccinia* were distinct species, and that it was impossible for barberry rust to turn into wheat rust. But he did not allow his inherited ideas to interfere with his studies, and in his second paper ten years later he was able to clear up these and many other erroneous beliefs. He introduced the culture method of study. He found that the rusts have a succession of spore-forms, and that *Aecidium* and *Uredo* are only form-genera. He reformed the terminology by using aecidiospore, uredospore, and teleutospore. Most important of all, he detected and demonstrated an alternation of hosts, for which he supplied the term heteroecism.

It is difficult to realize that the dawn of a recognition of the rusts as individual objects dates back scarcely two hundred years, before which time there were only superstitions and wild guesses as to their nature, and that a fairly comprehensive idea of the rusts covers little more than a quarter of this period, in fact only about sixty years. The shortness of this latter period was impressed upon me forcibly by a personal incident. While on a brief visit to Strassburg in 1888 I met Professor de Bary, then apparently in the prime of life, although it was only a few months before his untimely death. My own active participation in solving rust problems, therefore, overlaps that of de Bary, and in fact covers more than two-thirds of the whole 60-year period. It should need no argument to convince any one that the unfolding of knowledge regarding a group of organisms so large and complex as the rusts must have gained only fair headway after six decades, and that the opportunities for profitable research are still unlimited.

In selecting topics to represent the general progress of rust studies at the present time it will be well to begin at the beginning. And what is the

beginning in any subject in natural history? Clearly it is the recognition of individual kinds and their designation by names, permitting of record and discussion. It is the nomenclature question that is fundamental, for names are the worker's tools. It is a hackneyed subject and generally distasteful, but one that every writer necessarily deals with.

The rules of nomenclature should be the same for rusts as for any other group of plants. The methods of applying names and establishing their validity constitute the large part of the so-called rules of nomenclature, and such rules to be generally useful must be formulated and interpreted by an authoritative body, duly appointed and accepted by practically the whole mass of working botanists. Much progress has been made toward such a desirable situation, but we have not arrived, although I believe to do so will be eventually possible. Everyone who applies a name to a new species or desires to use correctly a name already established—if he has the good of the science at heart—must wish to feel that he is proceeding in a manner generally acceptable to his fellow workers. The name is a means of identification, and is not to be confounded with any question of classification.

In this connection I think it is pertinent to ask, what is a name, and how is it to be used to serve its purpose? Judging from current writings there must be much diversity of opinion. Is it the generic or the specific part that carries the essential feature of identification, or is it the combination? It is in fact all three, but in varying degrees of importance. The only unalterable feature is the specific part, which is presumably the first and oldest designation for the species, or one which for sufficient reasons has been accepted as a substitute. This essential part is validated when combined with some genus name to show in what group it belongs. The specific part in combination with any suitable genus constitutes the name; thus, *Puccinia glumarum*, *Dicæoma glumarum*, and *Pleomeris glumarum* are all correct names of a particular species of rust, and the choice lies with the user.

It has become customary, and in some cases is highly serviceable, to append a bibliographical citation to indicate the source of the original application, but such citation is no part of the name. It is a superfluous nicety to write *Puccinia glumarum* (Schmidt) Erikss. and Henn., except when required for the sake of uniformity, as there is but one rust called *Puccinia glumarum*. But if one desires to mention the common barley rust as *Puccinia hordei*, it is well to remember that this name has been applied to two distinct rusts. *P. hordei* Fuckel is the rust on wild barley occurring along the Pacific Coast and in Europe, but *P. hordei* Otth is the rust of the cultivated barley. Fuckel's name is the older one, and so

the common barley must take the next oldest name, *P. anomala*, unless the citation is used.

All this is highly elementary, but may be taken as an indication of progress in the application of Latin names to the rusts. Any serious attempt to establish the status of rust names dates back only a few years, and even now a large amount of work is needed to determine the correct application, especially of the older names. What would be highly serviceable is a comprehensive monographic treatise to be used as an authority, not a compilation like Saccardo's *Sylloge*, useful as that is, but a treatise more like Sydow's *Monographia Uredinearum*, with each name properly substantiated. This could be done by countries or large areas, and need not await a universal study. In the meantime sufficient progress has been made in providing authoritative treatises to permit omission in most instances of the obnoxious personal advertisement, as interpreted by C. G. Lloyd, except in formal taxonomic articles. At any rate it should be borne in mind that such an appendage is not a part of the name, and is only required for bibliographical identification.

This leads naturally to the question of new species. When I began my work with the rusts there was a certain amount of opprobrium attached to the publication of new species. The work of Mr. Peck and Mr. Ellis was rated as rubbish before the wheels of botanical progress. It was said that anyone might send a specimen and get a name for it by return mail, implying that species were manufactured for the asking. The taxonomic worker of today knows there was small ground for such accusation. It is to the untiring and conscientious devotion of Mr. Peck and Mr. Ellis and others of like spirit that we of today are enabled to have sufficient working material with which to untangle and arrange the hundreds of genuine species and their unlimited variations. In spite of the multiplicity of so-called species, both good and bad, there are still many true species and forms that await detection, and one need not go to the tropics to find them, as witness the admirable results achieved by Messrs. Fraser and Faull in Eastern and Western Canada.

The identification of species is not so simple a problem as it was twenty-five or thirty years ago. It is now realized that to describe adequately a species for purposes of full recognition it is necessary to take note of its several states or metamorphoses, and not only to describe each but show which, if any, are absent. Even then the task is not complete, for there are the hosts to be identified. But there are two phases in this situation: one relates to the application of the name, and the other to the question of classification.

It is manifestly impossible in many instances for the person who desires to describe a new species to have all the possible stages in the life-cycle at

his command. The more or less partial description must be drawn from the specimen in hand, and to that material the name is attached. It can be left to the future and to others to supply the missing items and to decide if the name has been antedated, or if the same or some other name has been applied to another stage of the same species. The *sine qua non* is an accurate description of a single definite object. To discourage the description and naming of forms thought at the time to be distinct species would be to lose to science much valuable data.

When it comes to the problem of classification, however, the subject of species and their names assumes a wholly different aspect. Let us illustrate with a comparatively simple example. *Puccinia andropogonis*, a common grass rust on the various species of *Andropogon* throughout North America, is known to develop its aecia on *Pentstemon* and *Comandra*, making two races, differing slightly in morphological characters. It is also probable, although not yet positively proved by cultures, that *Aecidium onobrychidis* on various leguminous hosts, and *A. polygalinum* on *Polygala senega*, bear the same relationship. There is not much doubt that other aecial forms which now bear independent names are also to be included. In the happy days of not very long ago all these forms could be treated as so many species; now, however, we are disposed to put them under one name. But our task with this one species is not completed when all the aecia have been rounded up and impounded. There are a number of correlated short-cycle rusts on the aecial hosts that undoubtedly belong in this group and have the same ancestry, such as *Puccinia pentstemonis*, *P. seymeriae*, *P. comandrae*, and possibly others. Now are all these forms, these so-called species, to go under one name, and constitute the variables of the true *Puccinia andropogonis*? That seems to be the conclusion according to our present tendency.

When such a diversified species—and there are many of them—is fitted into a natural classification, and we look for clues to relationship with other species, are we to seek them in the gametophytic or the sporophytic phase of the species? Some persons consider the teliosporic state of a species as the most important. But to do so one must omit from consideration the question of gametophyte and sporophyte and the influence of the host, for the telia of the long-cycle and of the short-cycle forms together embrace both sporophyte and gametophyte and the whole gamut of phanerogamic possibilities. If we select the sporophytic phase as the more important for indicating relationship, then only the telia of the long-cycle forms and their hosts are in evidence. But some botanists consider the gametophytic phase as the older and more primitive generation; they believe that it presumably antedates the sporophyte in evolution, and should therefore more definitely

indicate the course of phylogenetic evolution and the degree of relationship. This would make the aecia and their hosts, which are also the hosts of the correlated short-cycle forms, more important in tracing relationship and deciding position in the scheme of classification. Herein lies a puzzle no one has yet solved, a problem that lies at the very threshold of taxonomy for the Uredinales. This problem is the chief reason why our classification of the rusts is yet artificial and chaotic. In other groups of plants, morphological characters largely determine relationship; but in the rusts they are only of coördinate importance, and the bearing of the host must always be considered.

The most notable advance in rust studies during the last few years has been along the line of physiological specialization and breeding for resistance. This is a fascinating field for research, and fortunately has attracted many able investigators. The assiduity with which it is pursued springs from the same motive that fixed the attention of the ancient Greeks and Romans and of students all along the modern pathway from de Bary to Stakman—its economic importance. In the attempt to solve the very difficult problem of preventing financial loss from rusts, the science of uredinology has greatly benefitted.

The chief attack along this line has been made on stem rust, and the prospect is bright for a practical control of this greatest source of injury to crop production. It may not be possible to banish rust from our grain fields in the same degree that yellow fever has been wiped out in most regions where it once flourished, but it should be possible to restrict it to a negligible amount. In the meantime, while that desirable end is being attained, a vast amount of contributory information about the nature and habits of the rusts in general is being unearthed.

In the various lines of inquiry nothing more striking has come to light than the marvelous degree of specialization found in some of the cereal rusts. In the stem rust, *Puccinia graminis*, not only are there now recognized half a dozen or more varieties—or races as they are commonly called by mycologists—according to the genera or species of hosts harboring them, but each race may be subdivided into many physiological forms. In stem rust of wheat such recognized forms run into the scores. They are too numerous and too shadowy for names, and so are given numbers. Of course these numbered forms can be subdivided in some cases. The possible computation is startling. But if this can be said of stem rust, the mathematical possibilities of leaf rust mount into the empyrean, and it would seem to be a task for the gods to trace them. This comes from the fact that while stem rust has a single form of aecium on the barberry, leaf rust has many forms on highly diverse and distantly related hosts; conse-

quently the possibilities of specialization extend both to the aecial and telial sides of each race. The study of leaf rust has barely begun, and even the boundary of the species has not yet been determined, a species which promises by its amplitude and ubiquity to justify the early name of *Puccinia rubigo-vera*, the truly representative rust.

While it has been recognized from earliest times that rusts are spread from plant to plant by wind-borne spores, yet a direct demonstration and study of the method was not undertaken until 1881 when Marshall Ward, in India, caught the spores of the coffee-leaf disease on slides placed at varying distances and in different directions from the diseased trees. The use of sticky slides has been greatly extended in recent years by the aid of various kinds of spore traps, and the placing of these has taken into account vertical as well as horizontal distribution, since Stakman and his co-laborers introduced the use of the aeroplane. Probably the most successful and conclusive studies regarding the direction and extent of wind-borne spores are the result of recent stationary and aeroplane observations by the Dominion Rust Research Laboratory. These studies have supplied valuable data, and the long debated question whether the initial infection of grain fields in the north comes from locally overwintered urediniospores or from wind-borne spores from the south is again shifted to the latter view. The problem of the source of spring infection is of great economic importance and becomes more insistent as one proceeds northward; and nowhere can its solution be undertaken with such clear hopes of detailed success as in Manitoba. The solution, however, requires more extended and resourceful methods than at present employed; in fact nothing less than a well-directed and well-equipped army of observers will determine when and from where the winds begin to bring infection, although the question of overwintering of urediniospores could be pursued with less cooperation. As to the wind, it will be necessary to be wiser, at least in the spring time, than the men of old who could "not tell whence it cometh and whither it goeth."

In a recent study of geographical distribution of rusts in the arctic regions it has been plausibly suggested by Lind of Denmark that the winter winds playing over the surface of the snow, are the chief distributing agents. Possibly it may be true in a modified degree of the two most prominent cereal rusts in their northern limits, stem rust and leaf rust of wheat. These rusts may possibly develop strains with unusually hardy urediniospores that overwinter and are scattered by the wind. The thought is suggested by the recent discovery, again emanating from a Canadian experiment station, of a strain of stem rust with urediniospores having deeply colored walls and colorless contents. These are characteristics that accompany resting spores,

d in some manner have to do with the capacity of the spore to withstand toward environment. They are the most prominent features in the transformation of ordinary urediniospores into resting amphispores. But neither there is necessarily any relation between color changes and hardiness has not yet been studied experimentally.

Two other matters are suggested by the finding of deeply colored urediniospores with colorless contents. What is the chemical nature of the coloring matter of rusts? Very little is known about it, and few attempts at analysis have been made. It is said to be an oily substance. It is usually abundant in the young cells of the mycelium, primordia of the sori, and in the early stage of all sorts of spores, unless exception be made of the pycniospores, the so-called spermogonia. It may be a soluble carotinoid, as tests have indicated. It is usually some shade of yellow. It shows most of the oil globules and other food supplies within the cell, and may itself be a nutrient, and possibly associated with some vitamine. The coloring matter of the cell wall, on the other hand, is rarely yellow, but some shade of orange or reddish brown, and does not respond to the same reagents as that of the cell contents; neither is it available as a nutrient. Whether the basic structure of the color substance both in the cell contents and the wall is the same or not, is still a question of divided opinion awaiting demonstration.

Finding of deeply colored urediniospores with colorless contents was announced as a case of mutation—a color mutation, of course, like finding red ear of corn or a white-flowered bluebell. If a true mutation, as it seems to be, it is probably to be rated the first authentic instance observed among the rusts, although this method of variation has often been assumed to account for many morphological peculiarities. Probably the most important feature of the discovery lies in the emphasis to be placed upon the study of variation in pedigreed cultures, a field of research scarcely touched, and yet holding the greatest possibilities for an understanding of the origin and permanency of the diversified forms and potentialities among rusts, especially among such highly variable species as the stem and leaf rusts.

There are other ways of attacking the problem of rust variability arising from the single-spore culture method. The brilliant achievement emanating from this laboratory, the demonstration that pycniospores from two strains of sunflower rust or stem rust of wheat when intermingled induce aecia, and when kept separate do not, makes a fair beginning to the broad field of heterothallism and its possibilities. We may sometime know if the rusts have two sexes, or four sexes, or no sexes at all but only an assortment of nuclei or possibly of chromosomes. It may be that the way is open for a broader application of the principles of genetics than heretofore employed

in the solution of rust problems. Some aid is derived from cytology, especially in tracing the course of the nuclei during maturation and germination of the teliospore, as illustrated by the invaluable studies of Dodge. Whether the cytologists can ever utilize the behavior of the chromosomes in the rusts is doubtful, for at present they have not been counted with certainty, and their part in heredity is unknown.

When the problem of heredity is taken up in earnest in its broadest aspect, physiological factors such as susceptibility and immunity of the host, which now are chiefly uppermost because they have to do with economic problems, will take their place with many other features of the subject in which the morphological side will play a large part. It may be we shall find out why, or at least how, the pores in the urediniospores of a species come to be in the upper part of the spore, as in *Uromyces appendiculatus* on cowpeas, while at the equator in the same species on field beans.

In speaking of pores one is led to ask what determines the location of pores. It is easy to see how a pore at the apex of a teliospore that remains attached to the host, as in cereal rusts, is most efficiently placed to bring the germ-tube into the air for the distribution of the basidiospores. But why are the pores of urediniospores numerous and yet never at the apex of the spore, even when the spores are transformed into a resting spore and remain attached to the host? Although both urediniospores and aeciospores usually have more than one, often many pores each, in a few instances urediniospores have only one pore, as in some *Carex* rusts, and even then it is not at the apex. So far as I know, Dr. Buller is the only person who has made any suggestion regarding the pore situation. He expresses the opinion that with several pores the provision is made so "that at least one pore shall be on the side of the spore which happens to be turned towards the epidermis of the host-plant," and thus assist germination.

Every pore appears to be potentially capable of giving exit to a germ-tube, and often a germ-tube does appear from each pore. Do they all continue to grow and under favorable circumstances form mycelium? One would be led to infer as much from the manner in which observations on germination are usually presented. But a cell to flourish must have a nucleus, or conjugate nuclei as the case may be; and as only one such exists in each normal spore, only the germ-tube which receives it can continue to grow. And what determines the movement of the nucleus? Apparently the mechanical drift of the enclosing cytoplasm. Consequently the germ-tube that grows most rapidly from an aeciospore or urediniospore sucks in the nuclear mechanism along with the forward movement of the protoplasm and is fitted for continued growth, while the others must

perish. So we come around to agree with Dr. Buller that the most favorably placed pore "assists the process of germination" and the continued life of the individual rust.

There is something peculiarly intriguing in the relation of the uredinial generation to the existence of rusts in general. To be sure, it is the chief repeating factor, and in the case of cereal rusts and some others, particularly tropical species, it seems more aggressive and important than any other part of the life-cycle. Yet there are many species, all the short-cycle forms and many long-cycle forms like those of the genus *Gymnosporangium*, that flourish without uredinia. Some of these, for instance the hollyhock rust, are just as intensive and spread as readily as any propagated by urediniospores. Nevertheless the balance of facts that press upon one's attention force the impression that in some way the uredinia are an important factor, even an increasingly important factor, in the existence of rusts as a whole. Of the 60 unattached uredinia listed as species under *Uredo* in the North American Flora, all except four or five are tropical or subtropical forms, indicating the rarity of their telial forms. In temperate regions, as well as in the tropics, many species utilize only the uredinial stage, except under special conditions, for instance, the iris and blue-grass leaf rusts east of the Rocky Mountains; while most of the grass, sedge, and composite rusts show a great preference for uredinia under all climatic conditions.

One is almost led to think that the chief drift in the evolution of the rusts is toward the elimination of other spore-forms and the supremacy of the urediniospore. Such a culmination seems to have been reached in a violet rust of Southern Europe, *Uredo alpestris*, since all attempts to find any other stage have failed. Such an argument can be ably supported by appealing to the well-nigh generally accepted theory at present that the fern rusts are the modern representatives of the most ancient line of rusts. There are six genera of fern rusts known, all heteroecious or presumably so, and all with uredinia as a prominent generation. Moreover, two of the genera, *Urediniopsis* and *Hyalopsora*, have specially modified urediniospores fitting them for resting spores; that is, they possess amphispores in addition to the ordinary form. It is difficult to escape the conviction that in the uredinial generation the rusts are endowed with their strongest means for perpetuation. It is no wonder, then, that the economic necessity of fighting the spread and destructiveness of urediniospores is a battle royal.

On the other hand, however, when a species shows a tendency to shorten the life-cycle, it is the uredinial stage that is first omitted. When conditions for the growth of the rust are poor, as in over-ripened or poorly

nourished tissues, the production of urediniospores is restricted and may even be completely inhibited. This first came to my attention when I cultivated *Cronartium cerebrum* on young oaks in which the annual growth was completed. The aecial infection was followed directly by telia, and either no uredinia or only a few appeared. A similar procedure in other rusts, including those on cereals, has been observed and commented upon by several persons. The same general tendency is manifested when the growing season is shortened, or made less favorable by lower temperature or drought, as in high altitudes or latitudes. The same restrictions to growth may act to eliminate the aecia as well as the uredinia, and then a short-cycle form results. This transformation is observable in such species as *Puccinia podophylli* on the may-apple and *Gymnoconia interstitialis* on blackberries and dewberries. Autoecious species like these, however, in which the change from a long-cycle form without uredinia to a short-cycle form is readily observable, are said to be unstable or mutable. But the process can scarcely be called a mutation. Although such a change in heteroecious species has not yet been experimentally or directly observed, yet there are good grounds to believe that such is the method. Most of the 150 short-cycle species described in the North American Flora under *Teleutospora* and *Micropuccinia* have doubtless arisen from heteroecious species, and may eventually be so assigned. We are led to this belief, first, by finding short-cycle forms on aecial hosts, like *Puccinia andropogonis* in its short-cycle form on *Pentstemon* and *Comandra* and not on *Andropogon* or other grasses; and secondly by finding aeciospores and peridial cells in the sori of some short-cycle species, such as *Puccinia grindeliae* and *Uromyces scutellatus*, indicating that such species once had a distinct aecial stage.

Philosophizing upon the origin and phylogenetic development of the rusts has a number of important bearings. It develops a better understanding of the interrelationship of the different forms of rusts. It helps to point the way toward the completion of the life-cycle when only a single spore-form or generation is known. It tends to bring about a more consistent and stable classification. It enables the investigator of methods for economic control of the rusts to recognize whether he is working with the assistance of evolutionary processes or in opposition to them. The numerous theories regarding the course of rust development that have been advocated from time to time have in recent years been tested with a constantly wider knowledge of the less common rusts and also of a more detailed and searching study of the ones best known. Progress has been made all along the line.

If there is any one outstanding fact regarding the rusts that has received general acceptance beyond any shadow of doubt, and is confirmed

by experiment as well as observation, it is their strict parasitism. Repeated attempts to grow the rusts on artificial media, or even to accelerate the germination of the spores by using nutritive solutions, have met with uniform failure. But now word comes from Professor Fischer's laboratory in Bern that one rust has been discovered which shows a saprophytic characteristic. The spores of *Uromyces scillarum*, a not uncommon short-cycle rust on wild hyacinths in Southern Europe, have been found to germinate well in a decoction of horse dung, stewed plums, or garden soil, in fact much better than in water alone. This discovery should encourage further attempts to find some liaison in the rusts between parasitism and saprophytism.

There is one field of inquiry in which students of the rusts have made no progress, in fact have not even recognized the existence of the subject. "The discovery of things as they really are" is as important today as when Plato made the statement. What can be more reasonable and more necessary for the proper study of organisms of all grades from man to amoeba than the recognition of a distinction between normal, diseased, and deformed states. But in the study of the rusts all revelations of the microscope have generally been treated as normal, or variations of the normal, and the realms of pathology and teratology have been ignored or only vaguely hinted at, although in the study of the hosts there is no such confusion.

In the illustrations of germinating spores by the Tulasne brothers, Cornu, Carleton, Sappin-Trouffy, and other early and later botanists, all sorts of vagaries are figured from excessive elongation, unusual branching and abjunction of the promycelium, to misplaced and malformed spores. Such disturbances in normal behavior are largely traceable to the abnormal conditions under which germination is obtained. Rust spores germinate at their best for the most part in a saturated atmosphere, but the customary method of drop culture submerges them, and in this unnatural environment they show in their growth all sorts of ill effects. Such varied distortions are familiar objects, but the novelty of the subject lies in the interpretation. Carleton thought in 1886 that he had discovered a new method of germination, which might be utilized in classification. Clinton, in 1919, suggested that the rust derived an extended period for infection in some of the observed malformations. But whatever comments were made, there is no attempt to treat the matter from the standpoint of pathology. The recognition of disease in the host in connection with the rust is common, but never of disease in the rust itself.

When spores formed under normal conditions exhibit distortions in form or structure we have another situation which seems not to have been

evaluated correctly. The genus *Rostrupia* is a form of *Puccinia rubigo-vera* having more than two superposed cells in the teliospore. *Puccinia tomipara* is another form of the same species in which the teliospore is irregularly divided into a number of cells. The much-cited species *Puccinia vertisepta* is only a form with part of the teliospores having more or less oblique walls, a not unusual occurrence in many species. Such variations from the normal, which are properly disposed of under the caption teratology, having often been taken to indicate the course of development. Vuillemin, the able French mycologist, has made a study of the relationship of *Melampsora* and *Puccinia* based upon teratological material, and others have attempted to trace phylogeny by the same means. Such unusual spores are, however, only monstrosities and have little value in tracing either ontogeny or phylogeny, just as monstrosities among flowering plants have no taxonomic value. They may, however, become a hereditary feature, and be a characteristic of races, as in *Puccinia tomipara*.

Another field of inquiry that has not yet been explored is that of fossil rusts. About a half-dozen fossil forms, found in Europe, have been named, but all are of doubtless authenticity and add nothing substantial to our knowledge of early geological remains. There is every reason to believe, however, that valuable data will yet be derived when a suitable microscopical study of fossil structures is undertaken by persons equipped with detailed knowledge of mycelia, sori, and spores. There must also be a reasonable amount of taxonomic information. To know *Puccinia graminis* and rusts of that kind is not enough. Primitive rusts are likely to be of a different sort.

Having traversed by a series of saltations the ground covered by the announced topic of my discourse, starting in the pre-historical period, coming down to the present, and rebounding into the geological past, I now face about to see how much progress has really been made in rust study. Judging by comparison with the status of the subject at the beginning of the century there has been a marvelous advance, but in some directions more than in others. Morphology and life-histories have received much attention, but experimental development lags. We are just entering upon the highly promising study of heterothallism, mutations, and hybrids. The probable phylogeny of the rusts is still an open question. The nomenclature and classification of rusts are yet in a nebulous state, although progress has been made. Cytological methods have been utilized with great success, and yet only a few studies have followed the course of nuclear changes in pedigreed strains. The most conspicuous advance, and from the standpoint of the economist the most interesting, is that of specialization. I have not elaborated this part of the subject as much as its importance

apparently warrants, although I do not forget that I am speaking before a phytopathological society. My justification must lie in a premeditated intention to cover only the study of the rusts, and not that of the effect of the rusts upon their hosts, or of the protection of crops from their depredations. The great advances made in the application by physical science to practical requirements have never much outrun the theoretical and strictly scientific investigation of the underlying subject. I believe the same relationship will hold in mycology, and that the intimate study of the rusts, their history and development, will be an assistance in devising means for economic control.

TRANSMISSION OF THE VIRUS OF CURLY-TOP OF SUGAR BEETS THROUGH DIFFERENT SOLUTIONS

WALTER CARTER¹

In a previous paper by the writer² a method is described by which the leafhopper *Eutettix tenellus* Baker can be fed on solutions. That curly-top can be transmitted to healthy beets by non-viruliferous leafhoppers which had previously fed on diseased beet juice was also reported. The question naturally arose as to whether, by the use of this method, the virus can be transmitted through solutions on which the insect could easily be induced to feed. In the present paper are given data obtained from preliminary studies made in this connection. At the time of writing the paper previously mentioned, positive results had been obtained by the use of three sources of the virus, (a) one drop of diseased beet juice in 40 cc. starch solution, (b) viruliferous leafhoppers macerated in the solutions, and (c) a very large number of leafhoppers from a badly diseased field, fed on the solutions from which the virus was later taken by non-viruliferous leafhoppers and transmitted to beet plants. These positive results, which are listed here, were obtained rather late in the season of 1926, and subsequent unsuccessful attempts to repeat the experiments may have been influenced by the poor conditions obtaining during the fall and winter under greenhouse conditions.

VARIOUS SOLUTIONS USED

During the spring and early summer of 1927 a large number of trials was made to transmit the disease through a number of solutions, but the results were all negative. To illustrate the range of materials used in the feeding solutions, some of them are listed (table 1).

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The painstaking assistance of Mr. Van E. Romney on the routine labor and greenhouse work in connection with these experiments is very gratefully acknowledged.

² Carter, Walter. A technic for use with homopterous vectors of plant diseases with especial reference to the sugar-beet leafhoppers, *Eutettix tenellus* (Baker). Jour. Agr. Res. 34: 449-452. 1927.

TABLE 1.—*Unsuccessful attempts to transmit sugar beet curly-top to non-viruliferous leafhoppers fed on solutions on which viruliferous hoppers had fed previously or to which were added macerated curly-top leaves or macerated viruliferous hoppers*

Date, 1927	Source of virus	Solution
March 28	Feeding of viruliferous leafhoppers	Diluted beet juice
April 15	do	Steam extracted beet juice diluted to amber color with addition of 1% sucrose and 0.2% gelatin
April 15	do	1% sucrose and 0.5% gelatin
April 19	do	1% sucrose, 0.5% gelatin with trace of the enzyme zymon
April 19	do	1% dextrin in distilled water
April 20	One drop juice from diseased beet	1% dextrin in distilled water
April 20	Feeding of viruliferous leafhoppers	1% sucrose and 0.5% gelatin
April 20	do	1% sucrose, 0.5% gelatin with trace of the enzyme emulsion
April 25	do	1% sucrose and 0.5% gelatin, plus 2% by volume liquid invertase and 1 drop acetic acid (dilute)
April 26	do	1% sucrose and 0.5% gelatin, with 2% by volume liquid invertase
April 27	Macerated diseased leaves	1% soluble starch
April 27	Macerated viruliferous leafhoppers	do
April 29	do	do
May 5	Feeding of viruliferous leafhoppers	1% sucrose
May 9	do	1% sucrose
July 3	Macerated diseased leafhoppers	1% soluble starch
August 1	do	1% sucrose
August 1	Feeding of viruliferous leafhoppers	Quassia extract and 1% sucrose
August 6	do	Quassia extract

EXPERIMENTS ON TRANSMISSION OF CURLY-TOP IN 1926

Transmission of Curly-top by using crushed leafhoppers as the source of the virus³

The feeding solution consisted of 12 crushed viruliferous leafhoppers, 0.4 per cent soluble starch, and 40 cc. distilled water. Non-viruliferous leafhoppers were caged on this solution August 24, 1926, removed and caged

³ This transmission of curly-top using macerated viruliferous leafhoppers as the source of the virus was repeated later by Drs. Severin and Swezey, who used essentially the same technique in connection with another study. Acknowledgment is hereby made to these workers for their kind permission to refer to their confirmation of the writer's experiments.

on healthy beet plants August 31, and removed from the plants September 1. Curly-top symptoms appeared on two plants September 10 and on another September 14. One plant remained healthy. The same leafhoppers were caged on a second lot of four healthy beet plants for 24 hours on September 1. All these plants remained healthy. The leafhoppers were then caged on another lot of three healthy plants for 24 hours. These plants also remained healthy.

As a check on the preceding experiment, non-viruliferous leafhoppers were fed on a solution similar to that of the experiment except that crushed non-viruliferous leafhoppers were used. The non-viruliferous leafhoppers were transferred to healthy beet plants after feeding on the solution, but the plants remained healthy.

The fact is interesting that only plants in the first set of the experiment became diseased. The results are somewhat similar to those obtained when one set of viruliferous leafhoppers is transferred successively to a long series of plants. Under these conditions it often happens that the leafhoppers fail to transmit the disease to all the plants in the series.

Transmission of Curly-top by using the juice from diseased beets as the source of the virus

The feeding solution consisted of 1 drop of juice from a diseased beet in 40 cc. of a 1 per cent solution of sucrose. The solution was held for four days at 85° F., after which non-viruliferous leafhoppers were caged on it August 28, removed August 30, and caged on healthy beet plants the same day. Curly-top symptoms appeared 17 and 23 days later respectively.

Experiments in which maltose, dextrose, lactose, and raffinose were used in place of sucrose all gave negative results.

Since the transmission of curly-top by the use of juice from diseased beets as the source of the virus has already been recorded, the significance of this experiment lies in the fact that the virus was subject to high dilution.

Transmission of Curly-top by using, as the source of the virus, solutions on which viruliferous leafhoppers had fed

A feeding solution was used consisting of 0.2 gm. dextrose, 1.0 gm. maltose, 1.0 gm. sucrose in 250 cc. distilled water. A large number of viruliferous leafhoppers were caged with the solution for five days. Non-viruliferous leafhoppers were then caged with the solution and removed to healthy beet plants two days later. One plant out of five developed symptoms of curly-top 17 days later.

A second set of non-viruliferous leafhoppers was caged with the same solution and removed to healthy beets two days later. All these plants remained healthy.

Another feeding solution was then used consisting of the same sugars as for the preceding experiment with the addition of 1.0 gm. lactose and 0.5 gm. raffinose. Viruliferous leafhoppers were caged with this solution for five days. Non-viruliferous leafhoppers were then caged with the solution and removed to four healthy beet plants 2 days later, and allowed to feed for 24 hours. Three of these plants showed curly-top symptoms 12, 15, and 26 days later respectively.

The same leafhoppers were removed to a second set of healthy plants for another period of 24 hours. One plant showed curly-top symptoms 15 days later. Three other plants remained healthy.

The same leafhoppers were caged on a third set of healthy beet plants for 24 hours. One plant showed symptoms of curly-top 18 days later. One plant remained healthy.

A second lot of non-viruliferous leafhoppers was caged with the solution and removed to healthy beet plants after 2 days. Five hoppers were caged on each plant. One plant showed curly-top symptoms 51 days later. Three others remained healthy.

A third lot of non-viruliferous leafhoppers were caged with the solution and removed to healthy beet plants after 2 days. Three hoppers were caged on each plant. One plant showed symptoms of curly-top 70 days later and three plants remained healthy.

The period between exposure of the plant and the appearance of visible symptoms of disease ranged from 12 to 26 days for the first lot of non-viruliferous leafhoppers allowed to feed on the solution. The similar period for the second and third lots was abnormally prolonged, covering 51 and 70 days respectively.

Another feeding solution consisted of 1.0 gm. each of maltose, dextrose, and sucrose in 50 cc. distilled water. Viruliferous leafhoppers were caged with this solution for two days. Non-viruliferous leafhoppers were then caged with the solution and removed to healthy beet plants two days later. Two hoppers were caged on each plant. Two plants showed symptoms of curly-top. They had been abandoned as healthy after one month had elapsed since the plants were first exposed to the leafhoppers. The two diseased plants were not observed until after 39 days, but the incubation period was possibly a few days shorter than that.

Experiments run concurrently in which certain other sugars were used all gave negative results in curly-top transmission. These sugars were: Certo (fruit pectin), 1 per cent xylose, 4 per cent xylose, 1 per cent arabinose, 4 per cent arabinose. The solutions were prepared by mixing the sugars with 0.5 gm. potato starch in 250 cc. distilled water. Two lots of non-viruliferous leafhoppers were used in each experiment.

EXPERIMENTS IN CURLY-TOP TRANSMISSION DURING THE FALL OF 1927

A feeding solution of 1 per cent sucrose was used and a large number of leafhoppers from a badly affected beet field caged with it for six days. Non-viruliferous leafhoppers were then caged with the solution and removed to healthy beet plants two days later. Three hoppers were caged on each plant. One plant showed curly-top symptoms after 28 days and seven plants remained healthy. This experiment was then repeated. Two plants showed symptoms after 28 days and nine remained healthy.

Another feeding solution consisted of 1 per cent soluble starch in distilled water. Viruliferous leafhoppers were caged with the solution for five days. Non-viruliferous leafhoppers, after feeding on the solution for two days, transmitted the disease to one plant with an incubation period of 28 days. Seven plants remained healthy.

Two more experiments were made using the feeding solution consisting of 1 per cent sucrose in distilled water. Four positive cases of curly-top were obtained with incubation periods of 33 and 34 days. Seventeen plants remained healthy.

The experiments with the soluble-starch feeding solution were repeated. Three plants developed symptoms of curly-top after 33 days and 18 plants remained healthy. A second lot of non-viruliferous leafhoppers was caged with the solution and removed four days later to healthy beet plants. Five hoppers were caged with each plant. One plant became diseased 33 days later and five remained healthy.

The incubation periods of the disease obtained during the 1927 experiments were all considerably prolonged.

The non-viruliferous leafhoppers used in these experiments were taken from cages of reared material. These insects were checked for their freedom from disease by examining the healthy plants on which they were reared.

SUMMARY

1. Curly-top of sugar beets has been transmitted by leafhoppers which have fed on a suspension of crushed viruliferous leafhoppers in weak aqueous solutions of various sugars.
2. The disease has been transmitted by leafhoppers fed on diseased beet juice in a 1 per cent aqueous solution of sucrose.
3. The disease has also been transmitted by leafhoppers which had fed on a solution on which viruliferous leafhoppers had previously fed, the incubation periods in these instances, as a rule, being prolonged.

BUREAU OF ENTOMOLOGY,

UNITED STATES DEPARTMENT OF AGRICULTURE,

TWIN FALLS, IDAHO.

FILTRATION EXPERIMENTS ON CURLY TOP OF SUGAR BEETS

HENRY H. P. SEVERIN AND OLIVE SWEZY

INTRODUCTION

It has been assumed by some investigators that curly top of the sugar beet differs from other plant virus diseases because direct artificial inoculation of healthy beets with diseased beet juice failed to produce symptoms of the disease. Smith and Bonquet (4) failed to infect healthy beets by inoculating them with juice from diseased plants. Severin (3) demonstrated later, however, that juice pressed from the leaves and roots of curly top beets and inoculated into the crown of healthy beets caused typical curly top symptoms in 9 of 100 beets. Carsner and Stahl (2) confirmed the results obtained by Severin. Since the method of artificial inoculation of healthy beets with diseased beet juice produced such a low percentage of infection, it was considered impractical for use in experiments for determining the possibility of filtering the causal agent of curly top.

METHODS OF INFECTING BEET LEAFHOPPERS

Beet leafhoppers, *Eutettix tenellus* (Baker), have been observed, on rare occasions, with their mouth-parts inserted in drops of the clear, viscid liquid which sometimes exudes from diseased beet leaves. It was assumed that non-infective adults might be infected by feeding them on diseased beet juice absorbed by pith, sponge, wick, or cotton. Non-infective hoppers which had been fed by each method transmitted curly top to healthy beets. These methods of infecting leafhoppers gave such a low percentage of curly top that they were abandoned in favor of the two methods given below.

Carter (1) described a method of feeding leafhoppers on liquids enclosed by a fish swim-bladder sack suspended in a celluloid cage containing the insects. This apparatus was used in a modified form in the work here reported. A small cylindrical cage $5\frac{1}{4}$ inches high and $4\frac{3}{4}$ inches in diameter was used, which was provided on one side with a glass window 3 inches high by $2\frac{1}{2}$ inches wide. The other sides and top were covered with black sateen (Fig. 1). The leafhoppers, attracted by the light, would congregate on the sack or crawl up on the glass and in flying would often come to rest on the sack. This method was found most satisfactory in feeding the adults in the greenhouse. With the use of this feeding apparatus non-infective beet leafhoppers became infected and were able to transmit curly top to healthy beet seedlings.

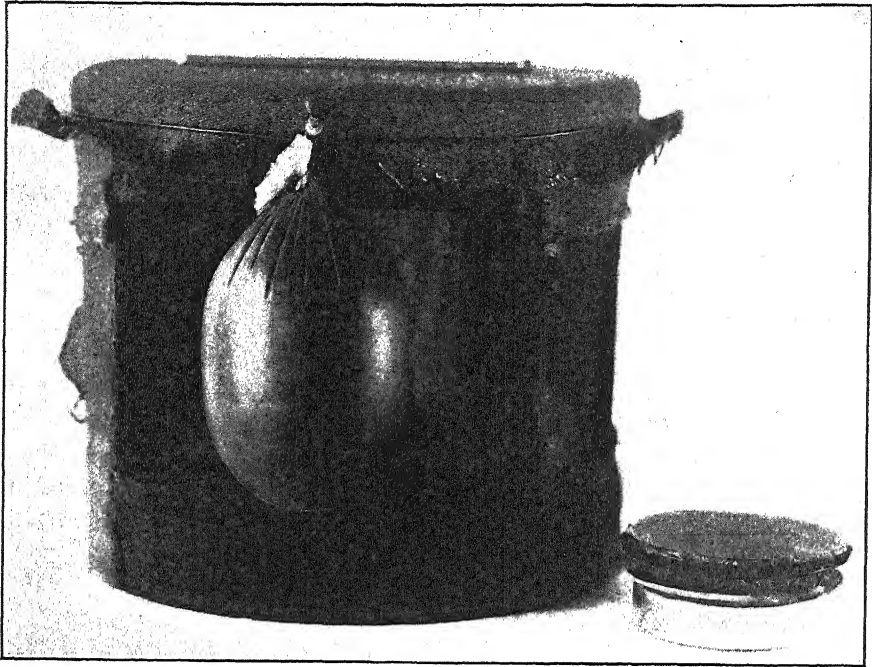


FIG. 1. Small cylindrical cage used in feeding beet leafhoppers on solutions in a fish swim-bladder sack or culture dish. The sack was suspended in the cage by a thread passed through a small hole in the denim and fastened to a long nail. In the photograph the sack was hung on the outside to show its size. When the culture dish was used it was placed directly in front of the window.

An equipment for feeding the nymphs was also devised by Carter, which was used in a modified form for these experiments. This apparatus consists of a small culture or "Esmarch" dish (50 by 10 mm.) containing the food solution and placed in the cage described above. The dish was covered with a fish swim-bladder membrane (Fig. 1), the bubbles of air below the membrane being removed by brushing lightly over the top with a camel's hair brush. Elastic bands held the membrane in place over the outside of the dish. The feeding equipment was placed in front of the glass in the cage.

Feeding Period.—When the feeding period is prolonged under high temperatures in the greenhouse, a high mortality of the adults occurs. During 1926 a series of experiments was undertaken to determine the shortest period required for large numbers of bugs to feed on beet juice. It was found that males which had been fasted overnight in the greenhouse and placed in a cool room the next morning would feed on beet juice in large numbers in

from two to four hours after it was offered to them. Root juice immediately after extraction turns black, owing to oxidation processes, and in a few days bubbles of gas form as a result of fermentation. It is probable that the toxic nature of the food caused the high death rate found among the insects when carrying on these experiments.

The feeding period of the nymphs with the use of food solutions in culture dishes varied considerably according to the time of day at which the experiment was begun. If a feeding experiment was started in the morning the bugs were transferred to healthy beet seedlings after sundown; if the experiment was set up during the afternoon the hoppers were removed during the next morning.

If too many nymphs or adults were used in the feeding experiments, the membrane became sticky owing to the minute droplets of liquid oozing through the feeding punctures, and the hoppers adhered to the membrane like flies on tanglefoot paper. When an experiment was conducted over a period of several days under high temperatures in the greenhouse the membrane was changed daily to avoid this.

The number of adults used with the sack method of feeding was about 60. In many experiments a second lot of 60 males was fed on the solution at the end of the first day when the first batch was removed, followed, in some cases, by a third lot at the end of the second feeding. The same procedure was often adopted in the feeding experiments of the nymphs.

At the end of the feeding period each lot of nymphs or males was divided among three healthy beet seedlings enclosed in cages, where they remained for a period of five days. At the end of this time the bugs were removed and the beets were placed in quarantine or insect-proof chambers, where they remained for a period of two months if curly top symptoms did not develop within the usual period of ten days to two weeks.

A number of experiments were conducted to test the effect of sunlight and shade on the feeding of leafhoppers on the virus of curly top, using juice from the leaves and roots of diseased beets. Six cases of curly top developed in 29 beets inoculated by the leafhoppers in sunlight and seven in 24 beets inoculated in the shade of a room. It was therefore decided to conduct all of the following feeding experiments on the tables in the greenhouse, as the insects are more active in sunlight than in shade.

METHODS USED IN FILTERING BEET EXTRACT

In the preparation of juice from curly top beets for filtration the sugar beets were ground to a pulp in a sterilized food chopper. The pulp was then collected in folded cheesecloth in a sterilized dish and the juice pressed out by hand.

In the experiments with macerated leafhoppers the steam-extracted beet juice was prepared by placing beet roots, cut in pieces, in an autoclave for a period of about one hour. Sugar solutions were prepared with sterilized distilled water, and added to this extracted beet juice. In all experiments reported in this paper a 5 per cent solution was used.

In all the filtration experiments the liquids were centrifuged from one to two hours. As a general rule the solution was first passed through a large, coarse Berkefeld candle (8 by 1 inches) and refiltered through small, medium, or fine cylinders ($2\frac{1}{2}$ by $\frac{5}{8}$ inches). With the use of this method little difficulty was experienced in filtering the juice from beet roots and culture media consisting of either a mixture of beet juice or steam-extracted beet juice and sugar solutions.

During 1927 a large number of filtrations were made using coarse (V), medium (N), and fine (W) Berkefeld candles or cylinders. The fine candles were tested with a small bacterium (*Bacillus prodigiosus*) so as to avoid the use of cracked or faulty candles with large pores.

FILTRATION OF ROOT JUICE

Experiments to determine the possibility of filtering the curly top virus were first undertaken by Severin and T. E. Rawlins during 1927. It was found that non-infective beet leafhoppers, after feeding on root juice, obtained from beets in an advanced stage of the disease and filtered through a coarse Berkefeld candle, transmitted curly top to healthy sugar beets.

During 1926 and 1927, root juice from 181 naturally infected sugar beets in an advanced stage of the disease, with wart-like protuberances on the lower surface of the leaves (Pl. XIV, B), was used in the filtration and control experiments. These badly diseased plants were selected in the beet fields of the Salinas and Sacramento valleys from May until October. Table 1 gives the results of the filtration of beet root juice with coarse, medium, and fine Berkefeld candles, also the unfiltered root juice used as a check or control.

As is shown in table 1, when non-infective beet leafhoppers fed on the filtered juice of diseased roots during the first day and then were placed on healthy beet seedlings, they transmitted curly top to 67.8 per cent of the plants used. Some experiments were continued for three successive days with a different lot of non-infective insects for each day. Those fed on the second and third days transmitted curly top to 26.6 and 7.6 per cent, respectively, of the healthy beet seedlings.

Filtration experiments were next conducted with root juice from beets showing the first visible foliage symptom of curly top, namely, the clearing or transparency of the minute veins (Pl. XIV, A, C). Infective beet leaf-

TABLE 1.—*The effect of inoculating sugar beets with curly top by means of leafhoppers fed on filtered juice from roots of sugar beets in an advanced stage of curly top*

Berkfeld candles used	Filtrate one day old		Filtrate two days old		Filtrate three days old	
	No. beets inoculated	No. beets infected	No. beets inoculated	No. beets infected	No. beets inoculated	No. beets infected
Coarse	26	18	21	9	7	1
Medium	15	13	12	2	3	0
Fine	15	7	12	1	3	0
Total	56	38	45	12	13	1
Percentage infected		67.8		26.6		7.6
Unfiltered check	17	9	9	3	3	0
Percentage infected		52.9		33.3		0

hoppers were fed on 24 beets for a period of two weeks and then the juice was extracted from the roots. These beets were divided into two groups: number 1, consisting of beets with an average diameter of 1 inch, each infected with 25 male leafhoppers; and number 2, of beets with an average diameter of 2 inches, each infected with 50 male hoppers. Further details of these experiments are given in table 2.

Some of the experiments were continued for three successive days as in the preceding group. It is evident from tables 1 and 2 that a rapid inactivation of the virus begins after the first day, as is shown by the percentages of curly top obtained on the three successive days in both filtered and unfiltered root juice. This inactivation of the virus is probably associated with chemical changes in the root juice, oxidation, and fermentation processes.

FILTRATION OF MACERATED INFECTIVE BEET LEAFHOPPERS

In the filtration of macerated beet leafhoppers, approximately 1,000 or 2,000 adults were used in each experiment, about 16 hoppers being used for each cubic centimeter of culture medium in the first two experiments and about 33 leafhoppers per cubic centimeter in the last two experiments. The insects were transferred from diseased beets to an empty cage and were fasted for a period of 24 hours. This was done to insure evacuation of the food in the alimentary canal, thus avoiding the possible transmission of unchanged diseased beet juice.

In the preparation of macerated, infective beet leafhoppers for filtering, large numbers of adults were captured with a pipette (Fig. 2) and put in small specimen vials containing equal parts of steam-extracted healthy root juice and a 5 per cent sugar solution. The bottle was shaken so that the wings became wet and the hoppers were unable to fly. The bugs were then dumped into a small mortar and ground with a pestle large enough to fill the lower portion of the mortar. The macerated insects were then poured into test tubes containing the desired amount of beet juice and sugar solution.

The culture medium containing the macerated beet leafhoppers was centrifuged for one hour and then passed through a fine Berkefeld candle. In most of the experiments half of the filtrate was fed to 60 non-infective nymphs during the first day, while the remainder was incubated for one day at room temperature and fed to another lot of 60 non-infective nymphs during the second day. The results are given in table 3.

It is noteworthy that the greatest percentage of infected beets in table 3 occurs in those inoculated by hoppers fed on the second day, but the number of experiments is too small to draw any definite conclusions. It is evident from these experiments that a filterable stage of the virus occurs in the beet leafhopper.

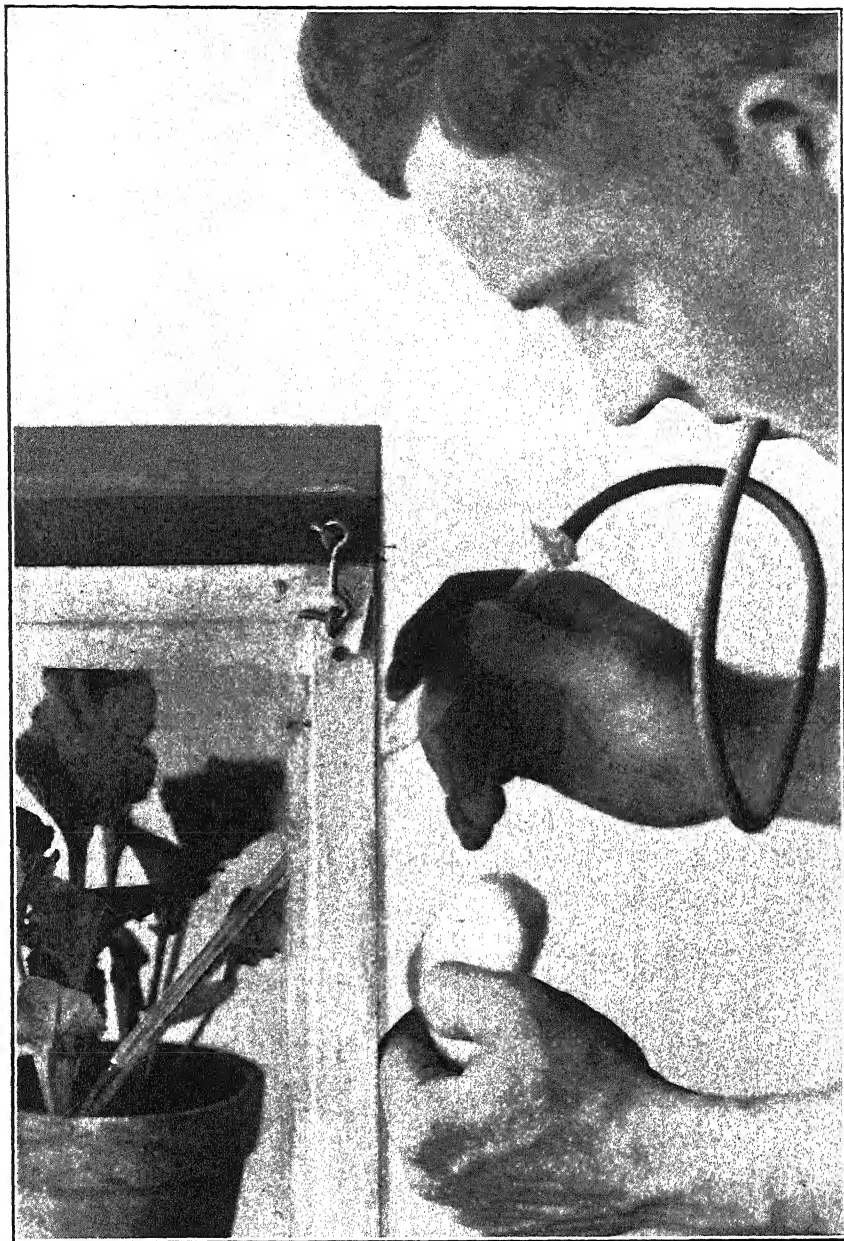


FIG. 2. Method of removing beet leafhoppers from a cage with a ten cubic centimeter pipette. By inhaling a breath of air through the rubber tube, the hoppers are drawn into the bulb of the pipette, and by exhaling the breath they are expelled from the pipette into a vial. A piece of silk bolting covers the opening between the pipette and rubber tubing.

TABLE 2.—*The results of inoculating healthy sugar beet seedlings with early top by means of leafhoppers fed on a filtrate of juice extracted from beets on which infective leafhoppers had been fed for two weeks.*

Berkefeld candles used	Filtrate one day old		Filtrate two days old		Filtrate three days old	
	No. beets inoculated	No. beets infected	No. beets inoculated	No. beets infected	No. beets inoculated	No. beets infected
Coarse ^a	3	3	3	1	3	0
Medium ^a	3	3	3	2	3	1
Fine ^a	3	1	3	0	3	0
Fine ^b	12	9	6	3	1	0
Total	21	16	15	6	10	1
Percentage infected		76.1		40.		10.
Unfiltered residue	6	3	3	0		
Percentage infected		50.		0		

^a Beets in this group had an average diameter of 1 inch, and each one was infected by 25 male leafhoppers.

^b Beets in this group had an average diameter of 2 inches and each one was infected by 50 male leafhoppers.

TABLE 3.—*The results of inoculating healthy sugar beet seedlings with curly top by means of nymphs fed on filtrates from centrifuged cultures of macerated leafhoppers,^a beet juice, and sugar solution*

Kind of sugar used in culture medium	No. hoppers in 1 cc. of liquid	Fresh filtrate		Residue after centrifuging		Filtrate after incubation for one day	
		No. beets inoculated	No. beets infected	No. beets inoculated	No. beets infected	No. beets inoculated	No. beets infected
Beet, commercial	16	3	1	3	2	3	3
Cane, commercial	16	3	0	3	2	3	1
Sucrose, chemically pure	33	3	2	3	3	—	—
Saccharose, chemically pure	33	3	0	3	2	3	3
Total		12	3	12	9	9	7
Percentage infected			35.		75.		77.7

^a The hoppers were collected from diseased beets and placed in an empty cage for a 24-hour fast before maceration.

SUMMARY

It has been found that non-infective beet leafhoppers when fed on root juice expressed from curly top beets in advanced and early stages of the disease and filtered through coarse, medium, and fine Berkefeld candles, transmitted curly top to healthy sugar beets. A rapid inactivation of the virus begins after the first day in both filtered and unfiltered root juice when exposed to the air through mouth-part punctures in the membrane.

Non-infective beet leafhoppers, after feeding on a culture medium in which infected leafhoppers were macerated, centrifuged, and filtered through a fine Berkefeld candle, transmitted curly top to healthy sugar beets. The infective beet leafhoppers were fasted for a period of 24 hours before maceration, so that the food in the digestive canal would be eliminated, thus avoiding the transmission of unchanged diseased beet juice.

It is evident from these experiments that a filterable stage of the virus of curly top occurs both in beets and the beet leafhopper which transmits it.

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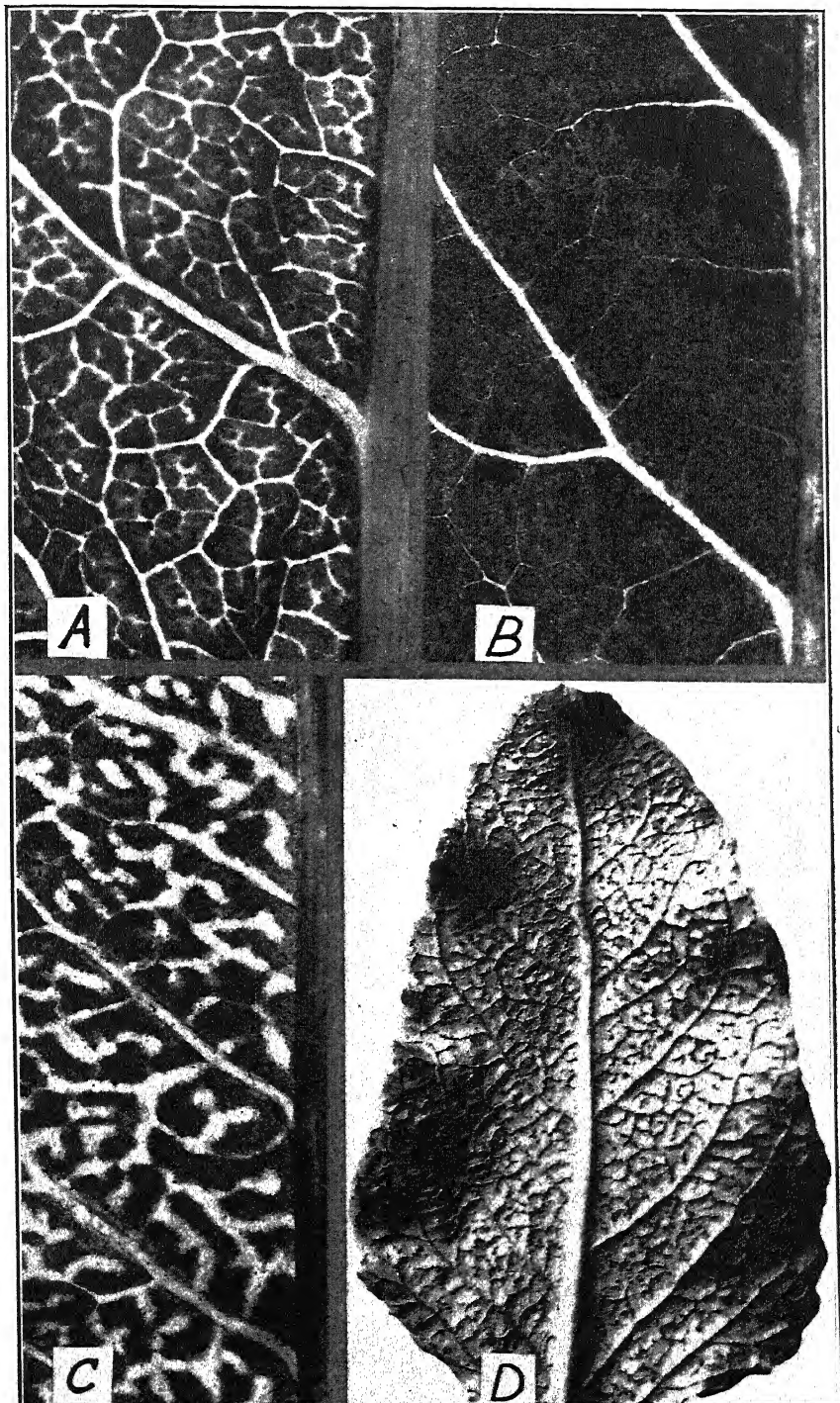
EXPLANATION OF PLATE XIV

A. Portion of youngest beet leaf affected with curly top showing early stage of transparent network of minute veins.

B. Portion of healthy beet leaf showing normal venation.

C. Later stage of transparent venation. The same magnification was used in figures A, B, C.

D. Beet leaf with small, wart-like elevations on the veins, which give the lower surface of the blade a roughened appearance.



PRELIMINARY NOTE ON SOME SEROLOGICAL STUDIES OF ASPERGILLI¹

TAKASHI MATSUMOTO

The object of the present investigation is to determine whether it is possible to differentiate any species of *Aspergillus* by means of serological methods. Most of the cultures of Aspergilli used in the present investigations were obtained from the National Collection of Type Cultures in London.

The morphology of the fungi was carefully studied, and they were arranged in groups according to the morphological relationships.

Group I.

Aspergillus niger, Strain A. Liquefies gelatin.

1. *Aspergillus niger* A, No. 1
2. do No. 2
3. do No. 3

Strain B. Does not liquefy gelatin, or only very slightly.

4. *Aspergillus niger* B, No. 1
5. do No. 2
6. do No. 3
7. *A. carbonarius*
8. *A. awamori*
9. *A. luchuensis*

Group II.

10. *Aspergillus fumigatus*, No. 1
11. do No. 2

Group III.

12. *Aspergillus glaucus*, No. 1
13. do No. 2
14. *A. herbariorum*
15. *A. amstelodami*

¹ This paper was presented at the annual meeting of the A. A. A. S. held at Nashville, Tennessee, 1927. The same article, accompanied by tables and more extensive discussions, will be published later.

Group IV.

- 16. *Aspergillus oryzae*
- 17. *A. parasiticus*

Group V.

- 18. *Aspergillus nidulans*
- 19. *A. versicolor*
- 20. *A. sydowi*

Group VI.

- 21. *Aspergillus ochraceus*
- 22. *A. tamarii*

Rabbits were inoculated, at intervals of 6 days, with increasing doses (5,000,000–160,000,000 spores) of the spore suspensions of the following six *Aspergilli*: (1) *A. niger* A, No. 1, (2) *A. niger* B, No. 1, (3) *A. carbonarius*, (4) *A. fumigatus*, No. 1, (5) *A. amstelodami*, (6) *A. glaucus*. The first two injections were given subcutaneously, and the subsequent eight inoculations made intravenously. The fungi were planted on ordinary agar slants and kept at 25° C. for one week. The spores were collected in sterilized physiological salt solution by scraping off with a platinum spud. These spore suspensions were centrifugalized, and supernatant fluids were discarded and replaced with sterilized physiological salt solution. The tubes containing those suspensions were left on a test-tube stand until the larger number of the clumps settled in the bottom of the tubes, and the supernatant spore suspensions were collected in separate tubes. The suspensions were heated for 45 minutes at 65° C. The number of spores in the inoculum was determined approximately by means of Thoma's counting chamber.

All the rabbits were bled from the marginal ear vein on the 9th day after the last inoculation. Sera were separated by pipetting from the clot after coagulation. They were always kept in the cold room, as the addition of a preservative is not advisable.

AGGLUTINATION TESTS

A rather extensive series of agglutination tests with homologous antigens as well as with heterologous antigens was made, but owing to the difficulty in making good spore emulsion the results were not satisfactory. A large number of the spores in normal sera settled within 30 to 60 minutes and formed a uniform layer on the bottom of the tubes in the same manner as in the immune sera; therefore no accurate reading as to the agglutinating

affinity could be satisfactorily made. In testing with anti-amstelodami serum against homologous antigens, I found a slight but distinct clumping of spores at dilutions up to 1:80 after one hour incubation at 57° C., while in the normal serum much slighter clumping was observed only at dilutions of 1:20 and 1:40. Nevertheless any cross tests proved to be unsatisfactory since the reading was rather unreliable in such a low titre serum as mentioned above.

PRECIPITATION TESTS

The precipitation tests were more satisfactory than the agglutination tests. A series of precipitation tests was carried out with filtrates of cultures grown in ordinary broth which had been incubated for 7 weeks at 25° C. Clear filtrates were easily obtained by passing the broth cultures through a filter-paper. If the filtrates were very acid, they were neutralized with KOH. All filtrates were diluted with physiological salt solution in the proportion of 1:1. For controls a salt solution and a normal serum were used.

As has been expected, the precipitation took place generally more extensively with the homologous antigens and next with the heterologous antigens of the same group. For instance, in the anti-niger A serum and the anti-amstelodami serum, a distinct precipitation was observed at the tested dilution of 1:40, and trace in 1:80; while with the heterologous antigens such precipitation, if present, was found only in 1:10 or up to 1:20.

In connection with this test it should be noticed that some slight precipitation was always found in the normal sera added to either one of the two filtrates of *Aspergillus niger* at the serum dilution of 1:10 and 1:20. No explanation as to this phenomenon can be properly made at this moment, as they are practically neutral. Moreover, no such precipitation was observed in the remaining filtrates.

It seemed advisable to repeat the same tests by using some extracts prepared from the organisms. Accordingly the following two techniques were employed.

1. Five grams of dried mycelium with spores, dried at 60° C., were thoroughly ground in a mortar and 100 cc. absolute alcohol added. They were extracted for 7 hours at boiling point in a reflux condenser. The extract was evaporated in order to be freed from alcohol, and subsequently saline slowly added. The solution was then passed through a filter-paper and finally through a Berkefeld filter.

2. Five grams of the dried organisms, dried in the same manner as described above, were thoroughly ground in a mortar and 100 cc. of 0.1 ethylene diamine solution added. They were put in an electric shaker for 4 hours, and then left on the laboratory bench overnight. The following

morning they were filtered through a filter paper and finally through a Berkefeld filter.

The results of the precipitation tests with these two extracts proved to be unsatisfactory, as the precipitation took place in no noticeable degree.

COMPLEMENT FIXATION TESTS

So far as my experiments are concerned, complement fixation tests appear to be most promising for differentiating our fungi, since the reactions are definitely observed in the higher dilutions of sera.

Technique. The reaction of the reagents and glasswares has been found to have an important bearing on the accuracy of the test. Acidity and alkalinity may give rise to false reaction which may be positive or negative according to the degree of acidity or alkalinity.

The reagents used in this experiment were as follows:

For fixation

- 0.5 cc. of immune sera at varied dilutions.
- 0.5 cc. of antigens in saline (1:200).
- 0.5 cc. of complement (1:10).

For hemolysis

- 0.5 cc. amboceptor, an exact concentration to be used is determined by means of titration.
- 0.5 cc. sheep red blood cells (1:20).

Immune sera were inactivated by heating for one-half hour at 56° C.

Antigens were prepared in the following manner. The fungi were planted on Czapek's solution agar and kept for one week at 25° C. The spores were collected in saline in the same manner as described above. To prevent the extraction from the media of substances that may render antigen non-specific, the spores were washed at least twice with saline. This was performed by centrifugalizing them and removing the supernatant fluids with a pipette. The spores were emulsified by addition of saline, in the proportion of 1 cc. of the spore mass to 200 cc. of the saline.

Freshly prepared complement of guinea pigs was used in a 10 per cent dilution made with saline.

Erythrocytes from sheep were used after they had been washed several times, and made up in a 5 per cent suspension with saline.

Amboceptor was obtained by successive inoculations of a rabbit with the sheep red-blood cells. The serum was heated at 56° C. for 30 minutes on three successive days. The balance of the hemolytic system was obtained by means of an amboceptor titration in the same method as generally ap-

plied for Wassermann test. The exact amount of amboceptor to be used was daily determined in this manner.

In the beginning of the work the fixation was accomplished by placing the test tubes in the incubator at 37° C. for one hour. Later I found that more reliable and complete reactions were obtained when the fixation was extended over night in the cold room. The following morning the sheep red-blood cells and amboceptor were added and incubated at 37° C. for one hour. The first reading was made after the incubation period of one hour, but the final reading was made after keeping the tubes in the cold room over night. For controls the following three tests were made: (1) saline instead of the immune sera; (2) saline instead of the spore suspensions; (3) normal sera instead of the immune sera.

In the majority of cases the immune sera were found to fix with the homologous antigens as well as with the heterologous antigens, but, as has been expected, the complement fixing power was always strongest when the immune sera were tested against the homologous antigens. For instance, in testing with the anti-niger A serum I obtained complete fixation up to 1:160, good partial fixation at 1:320, partial fixation at 1:640, and a trace in 1:1280. Practically the same extent of fixation was observed in the different strains of *Aspergillus niger*, thus showing no correlation between the serological behaviors and the biochemical characteristics as manifested by the liquefaction of gelatin. In this respect all the strains of *A. niger* studied were considered to be identical. In addition to this specific reaction the anti-niger A serum and anti-amstelodami serum have shown a distinct group reaction. In the anti-niger A serum *Aspergillus luchuensis* gave complete fixation at 1:160, good partial fixation at 1:320, and a trace at 1:640; *Aspergillus carbonarius* also completely inhibited hemolysis at 1:160, partially at 1:320, and a trace at 1:640; about the same result was obtained with *Aspergillus awamori*. In the anti-amstelodami serum, *Aspergillus glaucus* and *Aspergillus herbariorum* showed exactly the same complement fixing affinity; both gave complete fixation at 1:80, good partial fixation at 1:160, and partial at 1:320; whereas in the homologous antigen complete fixation was observed at 1:320, good partial fixation at 1:640, and partial fixation at 1:1280. Besides those reactions stated above these two sera, as well as the other sera, showed also some general group reaction against the remaining species, although the titre was very low with a few exceptions (mostly 1:10-1:40).

Anti-niger B serum, anti-fumigatus serum, and anti-glaucus serum also showed specific homologous reaction as well as group reaction to a certain extent, but titre was rather low as compared with the above two sera. This might be partly due to the characteristics of these fungi, but also due to the

individuality of the rabbits inoculated. A further investigation of this matter will be made later.

In view of the results obtained from the precipitation tests and the complement fixation tests, it may be considered that the sera contain in addition to specific homologous precipitins and complement fixing substances several groups of such substances which act on different antigens at the higher concentration. Therefore, if the titre of these sera is taken into consideration, these serological methods can be applied to identify or differentiate the fungi to a certain extent.

The writer is indebted to Dr. J. C. Ledingham and the other members of the staff of the Lister Institute of Preventive Medicine for many helpful suggestions.

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EFFECTS OF MOSAIC UPON THE CHLOROPHYLL CONTENT OF TOBACCO¹

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Although the lighter green color is a marked pathological symptom of many mosaic diseases, there are but few studies of the chlorophyll of mosaic plants recorded in the literature. Dickson² has reported an apparent reduction in the chlorophyll content of the lighter green areas of mosaic tobacco plants and a greater-than-normal content in the darker areas. Elmer³ has shown, in a quantitative manner, a similar condition in tobacco mosaic, and in addition has found a reduction of the green pigments in mosaic tomato leaves.

The purpose of this investigation was to compare the chlorophyll (a + b) content of the leaves of mosaic tobacco plants with that of the leaves of healthy plants grown under the same conditions. Normal and mosaic plants were compared, as to chlorophyll content, from the seedling stage to maturity. The effect of the mosaic disease upon the leaves at different positions on the stem was investigated. Determinations were also made of the effect of the disease upon older plants which had been infected for a comparatively short time. The total chlorophyll of a normal tobacco plant was compared with the amount of pigment in the entire leaf tissue of a mosaic plant.

The chlorophyll determinations were made upon Havana tobacco plants grown in the greenhouses of the Connecticut Agricultural Experiment Station during the period from November, 1926, to the following May. Most of the diseased material was obtained from plants which were infected in the seedling stage by rubbing the leaves with mosaic virus. In the case of smaller plants, from one to three leaves with the midribs removed were used for a single chlorophyll determination. With larger leaves, a rectangular area containing 60 sq. cm. was cut from one side of the leaf blade and used for chlorophyll extraction. Similar material was selected from normal and mosaic plants at the same time. The method of Willstätter as modified by Schertz⁴ was followed in the extraction of the chlorophyll. Quantitative determinations of the saponified green pigments, chlorophyll

¹ Contribution from the Osborn Botanical Laboratory.

² Dickson, B. T. Studies concerning mosaic diseases. MacDonald College, Quebec, Tech. Bul. 2: 1922.

³ Elmer, O. H. Transmissibility and pathological effects of the mosaic disease. Iowa State Coll. Agr. Res. Bul. 82: 1925.

⁴ Schertz, F. M. Bureau of Plant Industry, U. S. D. A., unpublished work.

a and *b*, were made with a colorimeter, using an aqueous solution of a weighed amount of purified chlorophyll, which had been saponified to chlorophyllins, as a standard for comparison.

Over one hundred chlorophyll determinations were made upon many plants during a period of 20 weeks after infection. These determinations were made consecutively over the entire period. The amount of chlorophyll in each determination was calculated upon a basis of 10 gms. of fresh leaves and upon 1 sq. dm. of leaf surface, although smaller amounts of leaf material were actually extracted.

A smaller amount of chlorophyll (*a* + *b*) in the mosaic plants, as compared with healthy material, was found at all stages of growth. The reduction was first apparent from 10 to 20 days after infection, when a significant difference of from 15 to 20 per cent was found. Later determinations showed that the chlorophyll content of mosaic plants was always less. The smallest reduction due to the mosaic disease was found at a period from 8 to 13 weeks after infection. At this time the chlorophyll content of the diseased material was only from 10 to 15 per cent less than that of normal plants. As the plants approached maturity an increase in the amount of chlorophyll in the normal plants was found. The determinations showed no corresponding increase in the mosaic plants.

TABLE 1.—*Chlorophyll content of mosaic and healthy tobacco plants*

Weeks after infection	Milligrams of chlorophyll				Mosaic Normal	
	10 gm. of leaves		1 sq. dm. of leaves		weight	area
	mosaic	normal	mosaic	normal		
1.5-3	13.3	15.7	2.7	3.3	0.84	0.81
5-7	16.3	18.5	3.1	3.7	0.88	0.85
8-10	16.3	18.5	3.1	3.5	0.88	0.88
10-13	16.6	18.5	3.3	3.8	0.90	0.86
14-17	16.1	18.4	3.3	3.9	0.88	0.83
18-22	15.9	20.1	3.3	4.8	0.79	0.71

The chlorophyll contents of healthy and diseased plants at various periods after infection are given in table 1. Each figure represents the average of several determinations made in a given period of from two to four weeks. The chlorophyll contents given were determined from all the leaves of the youngest plants, while determinations upon larger plants were made upon the leaves half way up the stems. In the last two columns of the table are the chlorophyll content ratios of mosaic to healthy material, which were obtained by dividing the number of milligrams of green pigment found in a given amount of mosaic leaves by the chlorophyll content

of corresponding healthy material. Figure 1 shows the lower chlorophyll content of mosaic as compared with healthy plants, from the seedling stage up to maturity.

In comparing leaves from different parts of the stem of large, nearly mature, mosaic plants with similar healthy ones, it was found that the greatest reduction of chlorophyll occurred in the younger leaves. Leaves of healthy plants near the tops of the stems contained 20.4 mg. of chlorophyll per 10 m. of fresh leaves, whereas similar leaves from mosaic plants contained only 17.3 mg. Leaves farther down the stems were found to contain less chlorophyll, both in the diseased and normal plants. In the older leaves toward the base of the plants the reduction caused by the mosaic was less apparent.

Infection of older plants with mosaic virus caused a decrease in the chlorophyll content of the leaves that developed after infection, as compared

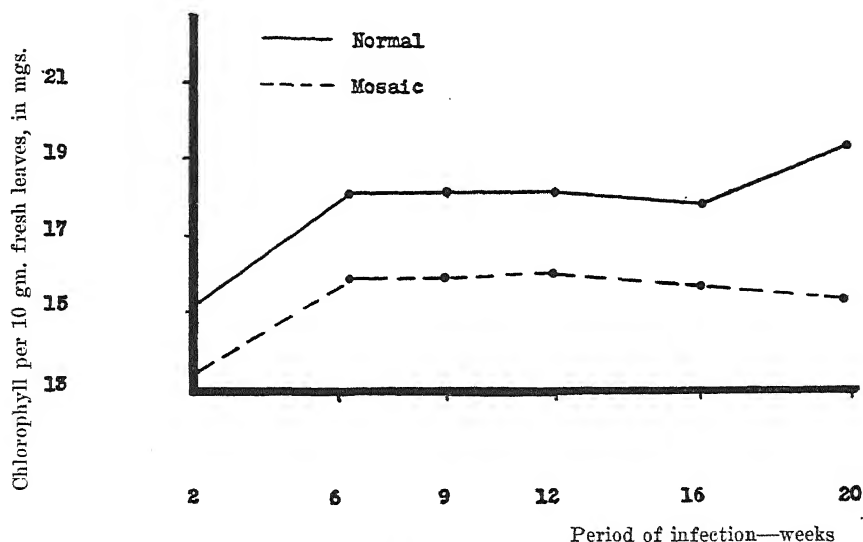


FIG. 1.—Chlorophyll content of tobacco plants throughout the growing period.

with healthy check plants. A slight loss of chlorophyll was found finally in the older leaves of the plants examined, although the older leaves showed no signs of mottling.

The total chlorophyll content of an entire mosaic plant was compared with the content of a corresponding healthy plant of the same age. The total area of these leaves on the normal plant was 89.7 sq. dm., as compared with 62.5 sq. dm. for the mosaic plant. The healthy plant was found to contain 359 mg. of chlorophyll, whereas the mosaic plant contained only 221

mg. In other words, the mosaic plant contained only 62 per cent as much chlorophyll as the normal plant. This difference was partly due to the somewhat stunted condition of the diseased plant and partly to the decrease of green pigment in a given amount of the mosaic material.

Aqueous solutions of the saponified chlorophylls from mosaic material have been observed under the colorimeter to be almost invariably of a yellowish green color as compared with the bluish green color of normal extracts. This change of color may be due to a greater proportion of chlorophyll *b*. Elmer⁵ has found this latter pigment to be more abundant in mosaic extracts.

SUMMARY

1. The mosaic disease of tobacco reduces the chlorophyll (*a* + *b*) content below that of normal plants.

2. A lower-than-normal chlorophyll content is characteristic of mosaic tobacco plants at all stages of growth.

3. The amount of green pigment in any mosaic leaf of a tobacco plant is below normal.

4. The chlorophyll content of young tissues is somewhat more seriously affected by tobacco mosaic than older tissues.

5. Older tobacco plants, after infection with mosaic virus, were found to contain subnormal amounts of chlorophyll, especially in the leaves that developed after infection.

6. The total chlorophyll content of mosaic tobacco plants is seriously reduced.

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⁵ Elmer, O. H. *l.c.*

AN ASCOMYCETOUS LEAF SPOT OF COWPEA

L. R. TEHON AND G. L. STOUT

Examination, during the course of our 1927 survey, of a field of cowpeas located near Huey, in Clinton County, Illinois, revealed a leaf spot of unknown cause. The spots on sample leaves taken from the field were found to bear perithecia, which, by their own characters, as well as the characters of their asci and spores, allied the leaf spot to similar diseases reported on alfalfa by Melchers (4) and F. R. Jones (2) as due to *Pleosphaerulina briosiana* Poll., on clover by Hopkins (1) as due to *Sphaerulina trifolii* Rostr., and on bur clover by Miles (5) as due to *Pseudoplea medicaginis* Miles. These repeated reports of similar fungi attacking and destroying the leaves of leguminous forage crops, together with the fact that the cowpea fungus possesses certain characteristics that differentiate it quite certainly from those previously noticed, lead us to give this brief account of a hitherto unknown cowpea disease.

The necrotic areas which appear as the result of the attack of this new fungus are exceedingly variable in size and seem to be influenced considerably by the resistance of the leaves, as indicated in the accompanying sketch (Fig. 1). The smallest spots, often not exceeding 2 mm. in diameter, are brown to tan, round to oval, and without a definite margin; spots from this size to 1 cm. wide are also brown to tan, round, and, in addition, nearly always have a distinct, rather wide, purple-tinted, dark margin; and the largest spots, reaching a width of 3 to 4 cm. or more, are of the same colors, except that the dark border is often very narrow.

Eventually the dead tissue in these spots becomes dry and friable and crumbles away, leaving large holes in the blade of the leaflet and deep semicircles in its margin. As the number of spots on a leaflet often is large, the destruction of leaf tissue is of considerable importance, and much damage to the plant must result. In the 5-acre field from which our samples and notes were taken, it was determined that 29 per cent of the plants bore this leaf spot, that on these plants 16 per cent of the leaves bore spots, and that as a result of the spots the surface of the leaves was reduced on an average, 13.5 per cent.

Even the smallest spots in the samples bore perithecia, and on the larger spots they were very numerous. Beneath the microscope they appear by reflected light to have burst the epidermis and to be protruding slightly, and when subjected to transmitted light they are seen to be mem-

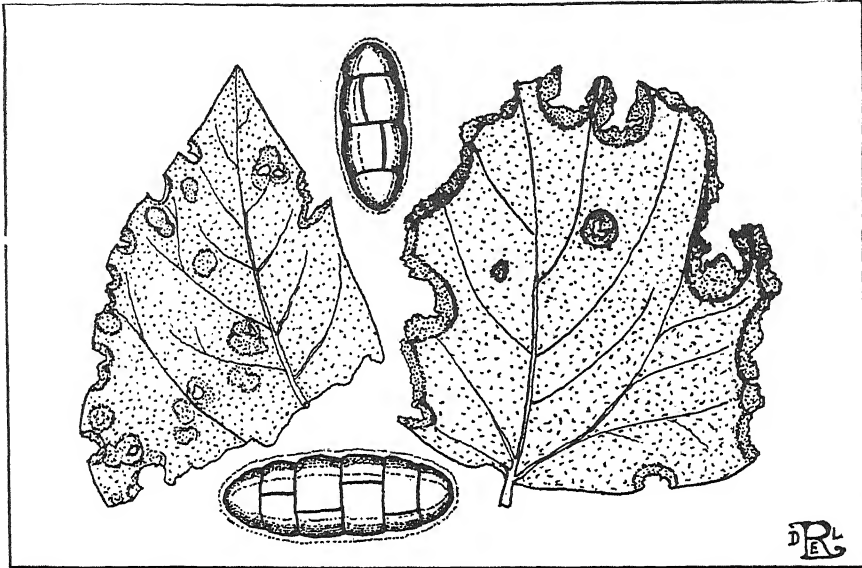


FIG. 1. Cowpea leaves attacked by *Leptosphaerulina vignae*, and two spores of the fungus.

branous, yellow-brown to brown, and spherical to markedly applanate. The ostiole is dark and often carbonous. The asci, when still immature, are distinctly saccate, with a stubby foot and a very thick apical wall; but as they approach maturity they assume a definitely oblong outline, and the thickened apex becomes nearly as thin as the rest of the ascus-wall. The spores in immature asci are hyaline and show no order of arrangement, though their final form is foreshadowed by the presence of three or more lateral septa and, rarely, one or two longitudinal septa; but at maturity they are dark brown, always muriform, and surrounded by a thin, colorless envelope that is, apparently, quite distinct from the spore-wall. There are no paraphyses.

As Miles (5) has noted in his paper on the bur clover malady, fungi of this type have been considered by von Höhnelt (7) as pseudo-sphaeriaceous; but Petrak (6), on the other hand, after more recent study, has contended that the species (*Pleosphaerulina briosiana* Poll.) upon which von Höhnelt based his *Pseudoplea* is in all respects truly sphaeriaceous and is closely akin to *Pleospora*. Though certainly suggestive, the distinctions upon which von Höhnelt based his mycological revisions have been so little approved that we hesitate to follow his generic segregation, preferring rather to locate the cowpea fungus as the second authentic species in Mc-Alpine's (3, Figs. 105-107) genus *Leptosphaerulina*.

Leptosphaerulina vignae n. sp.

Foliicolous. Causing more or less extensive necrotic areas which are brown to tan with a dark, purple-tinted margin, extend through the leaf, become dry and friable and crumble away. Perithecia innate, becoming erumpent, developed in and occupying the epidermal and palisade layers, membranous, light brown or yellow-brown by transmitted light, spherical to markedly applanate, 75–100 μ in diameter; furnished with a dark to carbonaceous, round, protruding, and very slightly raised ostiole; upper half often seeming to bear setae, these being in reality conidiophores upon which are borne spores of an *Alternaria* type, apparently a conidial form. Asci few, 4 to 6, rarely 10, in a perithecium, at maturity oblong and short stalked, with a not very evident thickening of the apex, chiefly 74 μ long by 25 μ broad but varying widely, 8-spored. Ascospores 30–40 \times 12–16 μ , arranged biserially, brown at maturity, chiefly with 3 lateral septa, but occasionally with 5, each of the two intermediate compartments always parted by a single longitudinal septum (spores of 6 compartments often with 3 and 4 longitudinal septa), provided with a hyaline mucilaginous envelope 1–1.5 μ thick. Paraphyses absent.

On *Vigna sinensis* Hassk. Huey, Clinton County, Illinois. September 5, 1927. The type specimen, on which the above description is based, is deposited with the mycological collection of the Illinois State Natural History Survey under accession number 20937.

Aside from the economic importance which this fungus may have, we regard it as of rather unusual interest mycologically, in that it appears to combine characters from three closely related ascomycetous families. In lacking paraphyses, in the characteristic shape and lateral septation of its spores, and in the thickened apex of the ascus, it relates itself quite evidently to *Sphaerulina* and *Pseudosphaerulina* of the Mycosphaerellaceae; in the further septation of the spore, its coloration, and the presence of pseudolocular perithecial partitions (regarded by Petrak as immature asci, but, as it seems to us, more probably undeveloped or rudimentary paraphyses) it shows characteristics commonly encountered among the Pleosporaceae; and in the one character of a gelatinous, hyaline spore envelope it is suggestive of the Massariaceae.

ILLINOIS STATE NATURAL HISTORY SURVEY,
URBANA, ILLINOIS.

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A CONSPECTUS OF NEEDLE RUSTS ON BALSAM FIRS IN NORTH AMERICA¹

J. S. BOYCE

The needle rusts of balsam firs form a group in which there has been considerable confusion. A study of herbarium specimens shows this, since collections under one specific name will in some instances have aecia on the current season's needles and in other cases on the needles of the previous season, or one year old. Field study has shown that the age of the needles on which aecia are found is of important diagnostic value, and not a single species is known in the Pacific Northwest with aecia normally on both the current season's and one-year-old needles, nor is any such occurrence elsewhere in North America reported in the literature. In fact, except for *Milesia pycnographis* (Bell) Arth., the aecia of which occur on needles from two to six years old, the aecia of these rusts are practically confined to needles of one season. Some of these rusts are frequently encountered in the Pacific Northwest, and in order to get a clear picture the present knowledge of their relationships was tabulated and is presented here with the hope that it may be useful to others (Table 1).

TABLE 1.—*A conspectus of needle rusts on balsam firs*

Uredinia and Telia		Pyenia and Aecia		Age of needles with aecia	References to notes
Rust	Host	Rust	Abies host		
<i>Calyptospora columnaris</i>	<i>Vaccinium</i> spp.	<i>Peridermium columnare</i>	<i>amabilis</i> <i>balsamea</i> <i>concolor</i> <i>fraseri</i> <i>grandis</i> <i>lasiocarpa</i> <i>magnifica</i> <i>nobilis</i>	current season	(1)
<i>Calyptospora</i> sp. ?	<i>T. macrophyllum</i>	<i>P. ornamentale</i>	<i>concolor</i> <i>grandis</i> <i>lasiocarpa</i> <i>nobilis</i>	1 year	(2)
<i>Melampsora humboldtiana</i>	<i>Salix</i> spp.	<i>Caeoma americana</i>	<i>balsamea</i> <i>concolor</i> <i>grandis</i> <i>lasiocarpa</i>	current season	

¹ In the preparation of this paper, publications of Dr. J. H. Faull and his students on the needle rusts of *Abies* have been freely consulted. Acknowledgment is also made to Dr. Faull for his helpful suggestions regarding the manuscript.

TABLE 1.—(Continued)

Uredinia and Telia		Pycnia and Aecia		Age of needles with aecia	References to Notes
Rust	Host	Rust	Abies host		
<i>Melampsorella elatina</i>	<i>Cerastium</i> spp. <i>Stellaria</i> spp.	<i>Peridermium elatinum</i>	<i>amabilis</i> <i>balsamea</i> <i>concolor</i> <i>grandis</i> <i>lasiocarpa</i> <i>magnifica</i> <i>nobilis</i>	current season	(3)
<i>Pucciniastrum abietichamaenerii</i>	<i>Epilobium angustifolium</i> and other species	<i>P. pustulatum</i>	<i>amabilis</i> <i>arizonica</i> <i>balsamea</i> <i>concolor</i> <i>grandis</i> <i>lasiocarpa</i> <i>nobilis</i>	current season	(4)
<i>P. epilobii</i>	<i>E. adenocaulon</i> and other species	<i>P. pustulatum</i>	<i>balsamea</i>	current season	
<i>P. myrtilli?</i>	<i>Vaccinium</i> spp.	<i>Peridermium</i> sp. nov.	<i>amabilis</i>	1 year	(5)
<i>Hyalopsora aspidiotus</i>	<i>Phegopteris dryopteris</i>	<i>Peridermium pycnoconspicuum</i>	<i>balsamea</i>	2 years	
<i>Milesia pycnographis</i>	<i>Polypodium vulgare</i>	<i>P. pycnographum</i>	<i>balsamea</i>	2 to 8 years	
<i>M. polystichi?</i>	<i>Polystichum munitum</i>	<i>P. rugosum</i>	<i>amabilis</i> <i>grandis</i>	current season	(6)
<i>M. kriegneri</i>	<i>Dryopteris spinulosa</i>	<i>P. kriegneri</i>	<i>balsamea</i>	current season	(7)
<i>M. marginalis?</i>	<i>Dryopteris marginalis</i>	<i>P. marginalis</i>	<i>balsamea</i>	current season	(8)
<i>Uredinopsis copelandi</i>	<i>Athyrium cyclosorum</i> , <i>Felix bulbifera</i>	<i>P. balsameum</i>	<i>balsamea</i> <i>grandis</i> <i>lasiocarpa</i> <i>nobilis</i>	current season	
<i>U. mirabilis</i>	<i>Woodwardia areolata</i> , <i>Onoclea sensibilis</i>	<i>P. balsameum</i>	<i>balsamea</i>	current season	
<i>U. osmundae</i>	<i>Osmunda</i> spp.	<i>P. balsameum</i>	<i>balsamea</i>	current season	
<i>U. phegopteridis</i>	<i>Phegopteris dryopteris</i>	<i>P. balsameum</i>	<i>balsamea</i>	current season	
<i>U. struthiopteridis</i>	<i>Woodwardia virginica</i> , <i>Struthiopteris germanica</i>	<i>P. balsameum</i>	<i>balsamea</i>	current season	
<i>U. macrosperma</i>	<i>Pteridium aquilinum</i> , <i>P. pubescens</i>	<i>P. pseudo-balsameum</i>	<i>amabilis</i> <i>grandis</i> <i>lasiocarpa</i> <i>nobilis</i> <i>venusta</i>	1 year	(9)

Note 1

Calyptospora columnaris causes witches' brooms on *Vaccinium*.

Note 2

Weir, J. R.: Observations on *Calyptospora columnaris* and *Peridermium ornamentale*. *Mycologia* 18: 274-277, Pl. 34-35. 1926. Weir suggests in this paper that the telial form of *P. ornamentale* is *Calyptospora* sp.

Peridermium ornamentale at present is undoubtedly a composite species, because *Peridermia* with aecia on the current season's needles and others with aecia on the one-year-old needles are so named. While the rust is listed here as occurring on four species of *Abies*, this is done simply because three of these hosts have been recorded elsewhere. In the Pacific Northwest the writer has found *P. ornamentale* with the characteristic laterally-flattened aecia on *A. lasiocarpa* only, and field study indicates that it may be confined to this host. At Government Camp, Oregon, for example, where *A. lasiocarpa* has been found commonly infected for several seasons, the rust has never been found on *A. nobilis*, although this host is more abundant there than *A. lasiocarpa*. As far as the age of the attacked needles is concerned, *P. ornamentale* is reported here only as it occurs on the original host species, *A. lasiocarpa*.

Note 3

Melampsorella elatina causes witches' brooms with deciduous needles on *Abies*.

Note 4

In a paper read before the International Botanical Congress at Ithaca, New York, in August, 1926, Faull pointed out differences in the period of time from inoculation to emergence of the aecia between *Pucciniastrum abieti-chamaenerii* and *P. epilobii*. Since nothing is known concerning these two species on their aecial hosts in the West, the known *Abies* hosts for *Pucciniastrum pustulatum* have all been listed under *P. abieti-chamaenerii*, while only *Abies balsamea* has been given under *P. epilobii*.

Note 5

Boyce, J. S.: A possible alternate stage of *Pucciniastrum myrtilli* (Schum.) Arth. *Phytopath.* 18: 623-625. 1928. In this paper it is suggested that the possible aecial stage of *P. myrtilli* is a *Peridermium* on *Abies amabilis*.

Note 6

Hotson, J. W.: Preliminary list of the Uredinales of Washington. *Pub. Puget Sound Biol. Sta. Univ. Wash.* 4: 273-391. 1925. On page 293 Hotson

suggests the possible connection of *Milesia polystichi* and *Peridermium rugosum*.

Although the aecia of *P. rugosum* occur on the current season's needles, they appear in the fall or occasionally in the late summer, when the needles are morphologically about one year old.

Note 7

In North American Flora 7¹⁰: 686. 1925, Arthur states that the aecia of *Milesia kriegeirina* occur on leaves two or more years old. In a letter of April 7, 1927, referring to the aecia of this species, J. H. Faull wrote, "They are found always on the needles of the current season." In Scotland, when the writer was shown a needle rust on *Abies pectinata*, which from field study seemed to be *M. kriegeirina*, the aecia were confined to the current season's needles.

Note 8

Milesia marginalis Faull and Watson was published by Faull in an abstract of a paper entitled, "Fern rusts I. The genus *Milesia*," which appeared in the Proceedings of the Royal Society of Canada, May Meeting, 1925. In North American Flora 7¹⁰: 686. 1925, Arthur evidently includes *M. marginalis* with *M. kriegeirina*, judging by the fern hosts given under the last-named species. In a letter of April 7, 1927, J. H. Faull wrote, "*M. kriegeirina* and *M. marginalis* are entirely distinct species, distinguished from one another by several well-marked points."

The rare bristlecone fir (*Abies venusta* (Doug.) Koch) is a new host for *Uredinopsis macrosperma*. The collection was made by H. G. Lachmund on January 6, 1926, at an elevation of 1500 feet on Pick Creek, headwaters of the Big Sur River, Monterey Co., California. F. P. No. 40467.

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PHYTOPATHOLOGICAL NOTES

Tomato Yellows or Tomato Curly Top.—The name of this disease first appeared in the literature as “summer blight,” then “yellow blight,” next “western blight,” and finally “western yellow blight.” The word “tomato” has been omitted in some of the names of this disease or the list would be longer.

In a number of recent papers, Shapovalov suggests that the name “western yellow blight” be changed to “tomato yellows” and this change was approved by the Pacific Division of the American Phytopathological Society¹ but has not been acted upon by the committee on nomenclature.

A large number of other cultivated plants have been shown to be naturally infected with curly top; and this name should be retained, if possible, for all of these crops. Bean curly top has already appeared in the literature, also squash curly top.

If the name of this tomato disease is to be changed, then “tomato curly top” should be used rather than “tomato yellows,” since “yellows” is already used with other insect-borne plant diseases. Yellows is also used with a considerable number of *Fusarium* diseases. The cultivated plants which are naturally infected with curly top would be segregated into a well defined group.

Kunkel² has experimentally transmitted aster yellows with the six-spotted leafhopper, *Cicadula sexnotata* (Fall.), to more than seventy species of plants in twenty-eight families. Kunkel³ has experimentally transmitted this disease to spinach and has proved that aster yellows is identical with white-heart disease of lettuce and with a previously undescribed disease of buckwheat.

Aster yellows made its appearance in California a few years ago, and it has been demonstrated by transmission experiments with *Cicadula sexnotata* that celery and lettuce are naturally infected with this disease. Celery has been experimentally infected with curly top by the beet leafhopper, *Eutettix tenellus* (Baker), but has not been shown to be naturally infected with this disease up to the present time. Spinach has been proven to be naturally infected with curly top but not with yellows.

Confusion may arise if the term “yellows” is used for certain plants infected with curly top, especially if a plant is naturally infected with the

¹ Report of the eleventh annual meeting of the Pacific Division of the American Phytopathological Society. *Phytopath.* 17: 745. 1927.

² Kunkel, L. O. Further studies on the host range of aster yellows (Abst.). *Phytopath.* 18: 156. 1928.

³ Kunkel, L. O. Studies on aster yellows. *Amer. Jour. Bot.* 13: 646-705. 1926.

two diseases.—HENRY H. P. SEVERIN, California Agricultural Experiment Station, Berkeley, California.

Grated Carrot Agar Favorable for Studies of Pythium.—It is sometimes difficult to obtain oospores from certain species of *Pythium* when they are cultured on the agars in common use in most laboratories. The writer has found this to be the case with a *Pythium* isolated from corn roots. A medium that has proved favorable for the production of oogonia and antheridia was prepared by adding grated carrots to water agar (17 grams of agar to one liter of water). In each test tube the grated carrot was placed to the depth of about 2.5 cm. and then approximately 10 cc. of melted water agar was added. The tubes were then plugged with cotton in the usual manner and autoclaved at a pressure of 15 lbs. for 30 minutes.

This medium was found to have the advantage of being sufficiently clear to permit examination of a plate under the microscope and yet to contain plant tissue without which some species of *Pythium* rarely fruit. Carrots were used because they are available throughout the year. Shredded fresh string beans were found equally satisfactory.—HELEN JOHANN, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture in cooperation with the Wisconsin Agricultural Experiment Station.

Disinfectants in Fire Blight Eradication Work.—Reimer¹, in Oregon, found that mercuric chloride was not uniformly effective as a disinfectant in fire blight eradication work, and adopted a mixture of this material with mercuric cyanide (1-500 each in water). Day², working under arid conditions in California, concluded that glycerine (three parts to one part of water) when added to the above mixture greatly improved its effectiveness. The writer has tested these materials in several experiments at Ithaca, New York. On cut twigs of vigorous apple trees mercuric chloride (1-500) injured the tissues to an average distance of 0.14 cm. from the wound, while mercuric cyanide (1-500) alone or in mixtures killed back the terminals 1.7 to 2.2 cm. One hundred and seventy twigs were treated in these tests.

Similar treatments were performed on wounds made with a cork-borer on trunks and branches of pear and apple trees. All of these wounds were inoculated with the fire blight organism from bouillon cultures. Mercuric chloride caused no measurable injury in these tests. In 2 of 35 wounds treated with this material, fire blight developed. Mercuric cyanide alone and in mixtures killed the bark to a distance of 0.2 to 0.4 cm. from the

¹ REIMER, F. C. A new disinfectant for pear blight. Calif. St. Comm. Hort. Mon. Bul. 7: 562-565. 1918.

² DAY, L. H. Experiments in control of cankers of pear blight. Phytopath. 14: 478-480. 1924.

margin of the wound, but not a single canker developed from the 59 wounds treated. In 6 of 24 inoculated but untreated wounds blight developed. The penetration of mercuric cyanide was apparently not appreciably increased by the addition of mercuric chloride or glycerine.

Mercuric chloride alone has been used in this state with apparently satisfactory results for many years. However in view of the available evidence and of the writer's own experience in practical eradication work, the mixture of mercuric chloride and mercuric cyanide in water is preferred for New York conditions until further data are available.—FRANK L. HOWARD, Department of Plant Pathology, Cornell University, Ithaca, New York.

The effect of washing the seed on infection of wheat by stinking smut.—During the course of some seed disinfection tests, it occurred to the writer to determine the effect of washing on the infection of wheat by stinking smut, *Tilletia levis*. Seed of an unknown variety, probably Fulcaster, was used. It was quite black with spores and contained 18 per cent of unbroken galls. A portion of the seed was placed in water and the floating trash and galls were skimmed off. It was then placed in a sieve under a stream of water from a tap, where it was washed for one hour. To the unaided eye the seeds were clean and bright after washing, but when viewed through the microscope many spores still remained on the brush ends of all seeds examined. Three brands of copper carbonate, designated as A, B, and C, were included in the test, also Bayer Dust and Semesan applied as a dust. The seed used for the dust treatments and for one of the check lots was hand cleaned before treatment, all galls being removed. The dusts were applied in excess and the excess removed with a sieve. An additional check lot from which the galls were not removed was also provided. The lots were seeded by hand on October 13, 1925, each occupying a single row 66 feet in length. They were harvested at maturity in June, 1926. Data on sound and infected heads are recorded in table 1. In view of the intensity of infection in the check lots the results obtained by washing appear very striking. The washing process gave as satisfactory control as the average of the dust disinfectants.

A second test of the effects of washing was made in the fall of 1926. The seed used was also quite black with spores of stinking smut and contained a number of unbroken galls which were removed by skimming. The lot subjected to washing was placed under the tap as in the tests of the preceding year and samples of 30 grams were removed after intervals of 15, 30, 45, 60, and 120 minutes. Two check lots, as well as a number of other treatments which will not be recorded here, were also provided. One check lot was

TABLE 1.—*The effect of washing and other seed treatments on infection of wheat by stinking smut*

Treatment	No. heads	No. infected	Percentage infected
Check, uncleaned	2270	1233	44.52
Check, cleaned	2040	800	39.22
Semesan, dust	1454	40	2.75
Bayer Dust	1523	34	2.23
Washed one hour	1602	13	0.81
Copper carbonate, Lot A	2000	4	0.20
Copper carbonate, Lot B	1613	2	0.12
Copper carbonate, Lot C	863	1	0.12

soaked 60 minutes in standing water, the galls being skimmed off, while the other was seeded dry without treatment. The lots were seeded by hand in 10-foot rows on October 2, 1926. Data obtained at harvest in 1927 are recorded in table 2. A marked reduction in percentage of infected heads in the washed lots will again be noted. Washing of 15 minutes reduced infection from 5.84 per cent in the dry check to 0.9 per cent, and additional washing resulted in further reduction.

TABLE 2.—*The effect of washing for stated periods on infection of wheat by stinking smut*

Treatment	No. heads	No. infected	Percentage infected
Check, soaked one hour	386	25	6.48
Check, dry	428	25	5.84
Washed 15 minutes	442	4	0.90
Washed 30 minutes	423	2	0.47
Washed 45 minutes	385	2	0.52
Washed 60 minutes	382	0	0.00
Washed 120 minutes	354	1	0.28

It seems evident that the reduced infection is to be attributed to the mechanical removal of spores by the stream of water. It could not be due to the action of water alone, since the lot soaked for 60 minutes in still water showed no reduction in infection. Such an interpretation is strengthened by the studies of Heald¹ on the relation of the spore load to infection. In these studies a minimum dosage of 0.5 grams of spores to 100 grams of seed was required to produce the maximum percentage of infected heads. A

¹ HEALD, F. D. The relation of spore load to the per cent of stinking smut appearing in the crop. *Phytopath.* 11: 270-278. 1921.

very considerable reduction of the spore load was accomplished by the washing process.—F. D. FROMME, Virginia Agricultural Experiment Station, Blacksburg, Virginia.

Prize Offered by the State of Rio de Janeiro, Brazil. The State of Rio de Janeiro, Brazil, has announced the offer of a prize of 100:000\$ (approximately \$1,200) to the scientist, either Brazilian or foreign, who prior to December 31, 1928, determines in an accurate and scientific manner the etiology of sugar cane mosaic and an effective method of combatting it, and presents the best thesis on this subject, this thesis to be published for the information of the interested public. Further information can be had from Dr. Eurico Teixeira Leite, Director, Instituto Fomento e Economia Agricola, Ministerio da Agricultura, Rio de Janeiro, Brazil.

BOOK REVIEW

Die Krankheiten der Obstbäume und Obststräucher. By E. Ewert. 2te neubearbeitete Auflage mit 63 Textabbildungen. 145 pp. Berlin. Paul Parey, 1926.

Professor Dr. Ewert has been the Director of the Botanical Experiment Station of the State College of Pomology and Horticulture at Proskau in Silesia, Germany.

In the first chapter a very short and popular survey of the system of fungi is given; some words are added on environmental factors and animal parasites.

The second chapter deals with disease control through fungicides, insecticides, insect traps, protection of birds and useful insects, and wound-cure.

Diseases common to all kinds of fruit trees are dealt with in the third chapter. It includes a discussion of conditions unfavorable for root development, root fungi, water rats (*Arvicola amphibius*) attacking the roots; frost, hail, hares, wood caterpillars, wood beetles, moss and lichens attacking or living on the stems and branches; dryness, silver leaf, chlorosis, caterpillars and beetles attacking the leaves and twigs; frost, caterpillars attacking the flowers; fruitdrop, cracking, sunscald, frost, and insect injury of fruits.

A similar arrangement is followed in the following special chapters: 4, on apple diseases; 5, on pear diseases; 6, on cherry diseases; 7, on plum diseases; 8, on peach diseases; 9, on apricot diseases; 10, on medlar diseases; 11, on quince diseases; 12, on currant diseases; and 13, on gooseberry diseases.

Besides some illustrations derived from Sorauer's *Handbuch der Pflanzenkrankheiten*, Ewert's booklet contains some good original photographs, e.g., the results obtained when currants are sprayed with bordeaux mixture as a protection against *Gloeosporium ribis*.

We see here, as in many German books, that discoveries made in other countries are not given the attention they deserve; the fact that silver leaf is caused by *Stereum purpureum* is not fully acknowledged. That *Nectria galligena* enters through leaf scars and scab wounds is not mentioned. Very valuable are the author's extensive observations on susceptibility of varieties. *Pinus cembra*, according to the author, is not a host for *Cronartium ribicola*. Carbolineum, which in Holland is one of the leading winter sprays, is not recommended in Germany on account of the inconstant composition. A spray calendar is not added.

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A STUDY OF THE HISTOLOGIC CHANGES INDUCED IN LEAVES BY CERTAIN LEAF-SPOTTING FUNGI¹

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INTRODUCTION

Just why many necrogenic fungi parasitic upon the leaves of plants make only a limited growth resulting in definite lesions known as "spots" has not been satisfactorily explained. So far as the writer is aware there is recorded in literature no comprehensive investigation on the subject. In the many papers dealing with leaf-spotting fungi certain susceptible reactions which may have some limiting influence upon the growth of the pathogene have occasionally been recorded. No doubt in many cases the reasons for such limitation of growth are purely physiological in character and have to do with the biochemical relation of pathogene and susceptible. In a few cases there have been reported certain histologic changes in the susceptible brought about as a result of the invasion of a necrogenic pathogene. Such histologic changes involve the revival of a meristematic condition in certain cells of the mesophyll resulting in the formation of new cells and an enlargement of these cells as well as of cells already in existence, with the ultimate production of a definite cicatrice separating the diseased portion from the healthy. Such a condition is reported as being more or less common in the leaves of higher plants when subjected to mechanical injury (Wylie, 21).

As a result of recently published work on the response of leaves to wounding and at the suggestion of Professor H. H. Whetzel, the present investigation was undertaken. The purpose of these studies has been to determine to what extent similar histologic changes are induced by leaf spotting fungi and whether such susceptible responses play a major rôle in the restriction of their necrogenic activities.

¹ This paper presents a part of the author's doctorate investigations on the pathological histology of leaf lesions. Grateful acknowledgement is made of the suggestions and criticisms of Professors H. H. Whetzel and A. J. Eames, under whose direction the work has been done.

It was deemed desirable to study as many different leaf-spot diseases as possible in order to arrive at any conclusions of general application. It was also necessary in each case to study a large number of sections to be sure of the true nature of the changes which take place. These changes were found to vary greatly with the proximity of the spot to veins and islet borders. In all probability the age of the leaf at the time of infection and also environmental factors play an important part in the type of susceptible reaction. In the case of wounding, the evidence of other workers goes to show that the moisture content of the atmosphere has a marked influence upon the type of healing tissue formed (Blackman and Matthaei, 1; Samuel, 17). It was also thought advisable to study, in a few cases at least, the response of the susceptible to artificial wounding.

All of the material used in these investigations was collected in the vicinity of Ithaca, New York. Specimens of the diseased material, together with representative slides, have been deposited in the herbarium of the Department of Plant Pathology, Cornell University.

HISTORICAL REVIEW

While there are numerous references in literature to the healing of wounds in woody stems and the histologic changes resulting in callus formation, there has been published comparatively little regarding the reaction of leaves to mechanical injury and still less with respect to their reaction to invasion by necrogenic pathogens.

Such references are scattered through various journals and in many cases are merely short paragraphs in a publication dealing primarily with the life history of the pathogen involved. This being the case, the author makes no claim to having exhaustively searched such literature but feels that the more important papers dealing with the subject have been consulted.

Bretfeld (2) states that in *Camellia japonica* wounds are healed simply by drying of the wound surface but that in *Bryophyllum* sp., for example, there is often formed a large and peculiar periderm and that in general the arrangement of this periderm is parallel to the edge of the wound.

According to Pierce (14) the shot-hole lesion in leaves of almond caused by *Cercospora circumcissa* is bounded by partially dead and thickened tissues.

Frank (5, p. 63-67) states that wounds made with a scalpel in the leaves of *Leucojum vernum* are healed by callus formation, while insect wounds in the leaves of *Cornus sanguinea* are healed by the production of wound cork. In the former case the healing tissue consists of a mass of enlarged cells with suberized walls while in the latter case a definite periderm is formed.

Massart (11) describes and figures a cicatrice in a wounded leaf of *Nuphar* sp. formed about the lacunae. He says that the age of the tissue is important in determining the extent of cicatrice formation.

Miehe (13), studying wounded leaves of *Tradescantia* sp., reports that the bared epidermal cells grow out and form a callus which at completion forms a cork layer.

Blackman and Matthaei (1) removed leaves of *Prunus laurocerasus* var. *rotundifolia* and placed the petioles in a beaker of water. When these leaves were wounded no healing tissue was formed. If, however, a sufficient number of cells were killed about the edge of the wound, an abscission layer was formed. The wounded portion eventually dropped out and the walls of the callus cells became cuticularized. Leaves wounded on the plants by cutting formed an abscission layer very quickly. The wounded portion did not fall out but division of the newly formed cells continued until a periderm of several layers of cork resulted.

Van Beusekom (18) noted that insect wounds on the tips of leaves of *Gnetum gnemon* brought about the formation of adventitious buds, and when the swollen tips were sectioned they showed multiplication and enlargement of the cells of the spongy parenchyma.

Wyneken (24) studied wound reactions in leaves in a large number of species of the higher plants and found that in some species a definite wound cork was formed. In such cases the outside layer of cells always had suberized walls, while in some cases lignification appeared in the walls of those cells lying next the suberized layer. In other species a callus composed of irregular, thick-walled cells was formed about the wound.

Higgins (7), working with *Coccomyces* sp. on species of *Prunus*, found that there was considerable variation in the deciduous character of the lesions in different species and even in the same species. He states that the separation is due to the abrupt enlargement of a layer of cells at some distance from the ends of the mycelium. The enlarged cells lose their chloroplasts and nuclei, and only a thin layer of protoplasm lines the walls.

A definite case of a barrier being laid down is given by Hesler (6), who worked with *Sphaeropsis malorum* on leaves of *Pyrus malus*. Where the lesion is not bounded by veins it is surrounded by a plate of cells which limits, for a time at least, the advance of the pathogene. The apparent stimulation results eventually in hyperplasia of the spongy tissue. The diseased cells gave a positive test for suberin.

Wylie (21), who severely wounded leaves of several species of higher plants, found that healing takes place quickly and the leaf continues to function. In a later paper (22), he discusses the structure of the healing tissues in species of widely different habitats. He found that this tissue is largely

formed by the development of new cells resulting from mitoses which establish walls parallel to the wounded edge of the leaf. All cell-layers of the blade share in this work, including the epidermis in case its cells are large. In a still later paper (23) he describes the healing process as embracing first the formation of a pseudo-cicatrice as a result of the death and collapse of exposed cells along the wounded edge, which is followed by the formation of the cicatrice proper.

Woit (20) studied wounded leaves in a number of species belonging to different families of the higher plants and found that many cells in full grown leaves are stimulated to division and new growth. Cells of the collenchyma, conductive tissue, and hairs are often active in the wound reaction. In a moist atmosphere callus is formed, while a freely exposed wound usually develops wound cork only.

In a study of *Prunus amygdalus*, attacked by *Clasterosporium carpophilum*, Samuel (17) found that the infected tissues in young leaves are invariably abscised. In the older leaves an abscission layer is formed but the infected tissues do not always fall out. The abscission layer begins with the swelling of cells in a narrow zone at some distance from the margin of the invaded area. Both palisade and spongy parenchyma cells are involved and complete occlusion of the intercellular spaces results. Where the invaded portion is abscised, suberization of the walls of the cells along the absciss line occurs soon after the cuticle ruptures. Subsequent divisions of the meristematic layer result in the formation of a few layers of brick-shaped cells which become suberized and slightly lignified. Where abscission does not occur the initial changes are similar, but in the later stages there is lignification of the walls of the cells on the inner side of the occluded zone and a suberization of the walls of the meristematically formed cells.

McWhorter (12, p. 19-21) describes the formation of a meristem in the leaves of *Carica papaya* infected with *Cladosporium papayae*. This meristem acts like a cork cambium, cutting off cork cells. The fungus is able to penetrate these cork cells to a limited extent.

It is evident from these records that mechanically wounded leaves of many plants respond by histologic modification of adjacent tissues. They also indicate that, at least in certain fungous diseases, similar healing-out modifications take place in the tissues about the lesion. However, one would be led to conclude from the very limited number of such cases recorded that such a response to the activity of pathogenes is relatively rare.

METHODS

As the material was collected small segments were removed from the diseased leaves by means of a razor blade. These segments included both

diseased and healthy tissue. When the lesions were small the portion removed from the leaf included the whole of the necrotic area. In the case of the larger lesions this procedure was not possible, as the size of the segment was necessarily limited by the difficulties of fixation. As far as possible only mature leaves with well developed lesions were used in these studies, several segments being taken from each of a number of infected leaves.

Immediately the segments were removed from the leaf they were placed in shell vials containing medium chromo-acetic fixing solution (3). In the case of some material, a vacuum-pump was used to insure rapid removal of air from the tissues. Other material was merely forced under the liquid by means of a wad of cheesecloth placed on top of the segments in the vial. The killing was perfectly satisfactory by both methods. The material remained in the fixing solution for from 24 to 36 hours and on removal was washed in running water for an equal length of time, after which it was imbedded in paraffin (53°-55° F.) in the usual manner.

Whenever time would permit, freehand sections were also made of the fresh material. At this time studies were made on the extent of the mycelial development, and microchemical tests were applied to determine the nature of the changes in the walls and contents of those cells lying at or near the edge of the lesion. In the study of freehand sections of freshly collected material cotton blue was used as a mycelial stain. This proved satisfactory in some leaves but was not so effective with others owing to the heavy staining of the chloroplasts. Tests for lignified tissue were made with phloroglucin and hydrochloric acid. The presence of cutin and suberin was determined by staining with Sudan III. This was done by boiling the sections in a drop of stain on the slide, washing in water, and mounting in glycerin for examination. The ferric-chloride test was used to determine the presence of tannins.

These tests were repeated with sections made from material imbedded in paraffin. In testing for suberin it was not possible to boil the stain on the slide after the sections had been attached with albumen fixative. Good results were obtained by placing the slides in a Coplin jar containing the stain and keeping this at a temperature of 40° C. for 36 hours. The slides were then thoroughly rinsed in water and mounted in glycerin for examination.

In addition to cotton blue as a mycelial stain, both Durand's (4) and Vaughan's (19) staining methods were used. None of the methods mentioned proved entirely satisfactory in all cases. For the purpose of general histologic studies, sections were stained on the slide in Delafield's haemotoxylin; safranin; Delafield's haemotoxylin and safranin combined; and in

crystal violet combined with erythrosin (8). The latter combination gave a beautiful contrast and was particularly valuable in detecting lignified tissue. In many cases it also proved valuable in staining the mycelium.

Some of the plates were prepared with the aid of a camera lucida, this being used merely to get the proportions and the bare outline of cells. The remainder of the plates were prepared by inking in the cell walls on an enlargement made from a photomicrograph. The enlargement was then treated with a solution of potassium cyanide, followed by a solution of iodine-potassium iodide, after which it was thoroughly washed and dried. This treatment removed the image and resulted in a plain black and white drawing. With both methods the details were filled in freehand. In all cases the details shown in the plates represent the author's interpretation of the condition existing in the cells as gathered from the study of a large number of sections stained in various ways.

Where artificial wounding was practiced the wounds were made on mature leaves by means of a common leather punch making a circular hole 2 mm. in diameter. In this way a clean-cut wound was obtained. As far as possible, care was taken to avoid cutting into or near the larger veins. Several wounds were made in a single leaf, the number depending somewhat upon the size of the leaf. The wounded leaves were allowed to remain on the plant for a period of from 18 to 21 days. At the end of that period they were collected and segments removed, treated, and studied in the same manner as those from diseased leaves.

PRESENTATION OF DATA

The results of the present investigation are presented under two headings based on the type of histologic changes which result from infection by the various pathogens on the susceptes dealt with in this paper.

In practically every case the leaf-spot lesion consists of a clearly defined central dead area surrounded by a zone of varying width and coloration in which the cells are still alive but diseased. Following the terminology used by Whetzel in his mimeographed text on symptomatology, this central region will be designated the "holonecrotic" region or area, while the marginal zone will be referred to as the "plesionecrotic" zone or region.

Unless otherwise stated, the mesophyll cells are of the usual thin-walled type containing nuclei and chloroplasts in a peripheral layer of cytoplasm. The epidermal cells also have a thin layer of cytoplasm about the walls, and nuclei are present.

*Diseases in which a Definite Cicatrice is formed
about the Margin of the Lesion*

Three species of *Coccomyces* and one species of *Mycosphaerella*, all of which are parasitic on members of the family Rosaceae, together with one species of *Cercospora* parasitic on *Beta vulgaris*, were found to cause histologic changes of the type under discussion.

The term "cicatrice" as used in this paper is applied to a band of healing tissue about the lesion or wound, the cells of which have been derived by division and enlargement of the mesophyll cells. The epidermal cells are also frequently involved. This cicatrice ordinarily consists of two parts, the outer part which is a typical wound periderm, and the inner part which lies between the wound periderm and the normal tissue. This latter part is composed of somewhat modified cells evidently not derived from the activities of the wound phellogen.

Lesions produced by Coccomyces prunophorae Higgins on Prunus domestica L. In the healthy portions of the leaf of *Prunus domestica* the upper epidermal cells are large and somewhat irregular in outline. Both the inner and outer walls are convex but this is more marked in the case of the inner walls. The outer walls are noticeably thickened as are also the radial ones, while the inner are comparatively thin. Except that the cells are somewhat smaller, the structure of the lower epidermis is very similar to that of the upper. The palisade parenchyma is well-defined and consists of two or even three layers of elongated narrow cells. In a cross-section of the leaf the upper layer appears unbroken by intercellular spaces. The lower layer, however, is less compact, and intercellular spaces are evident between many of the cells. The cells of the spongy parenchyma are more or less irregular in size and shape and are rather loosely arranged. In the immediate vicinity of the larger veins the palisade parenchyma is replaced by enlarged, circular to irregularly shaped, thick-walled parenchyma cells, while the region of the spongy parenchyma is occupied by the principal bundle tissues with strengthening cells below. Where smaller veins are present the palisade parenchyma is still intact, but that portion of the spongy parenchyma not occupied by the bundle is filled with somewhat small, thick-walled parenchyma cells.

The necrotic lesions caused by the fungus vary considerably in size and appearance depending upon the age of the lesion and the extent of the infection. The smaller lesions are more or less circular in outline, the dark-brown holonecrotic area being surrounded by a narrow yellowish-green plesionecrotic zone. Where the lesions are numerous they frequently coalesce to form large, irregular areas. In the older spots the diseased portion

may drop completely out, leaving a clean-cut margin. This margin may or may not be thicker than the normal leaf.

A cross-section made through one of these lesions reveals the presence of a very definite cicatrice at the margin (Fig. 1). Throughout the whole

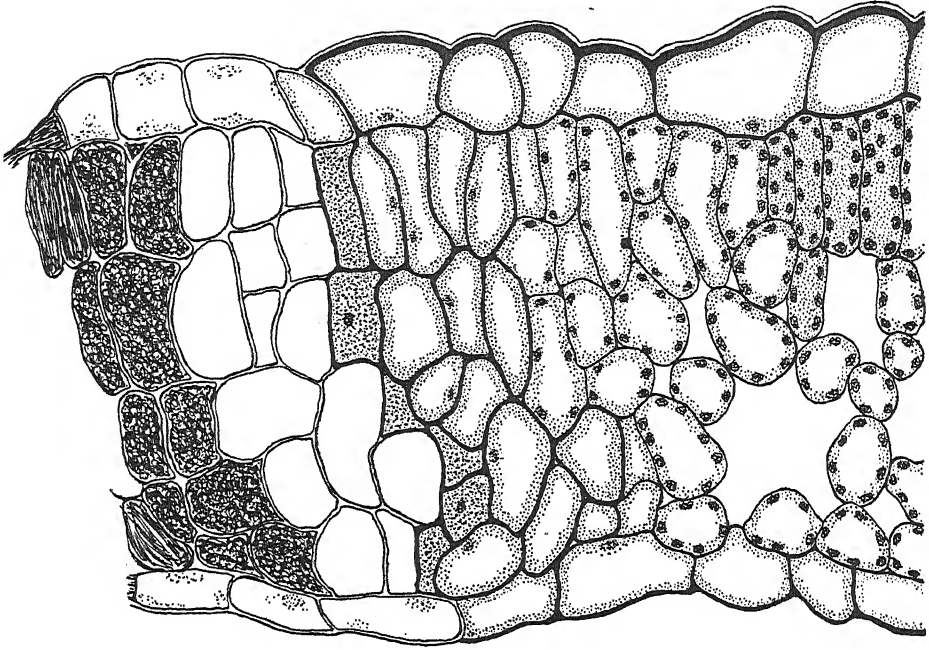


FIG. 1. Cross section through the edge of a lesion on a leaf of *Prunus domestica* caused by *Coccoomyces prunophorae*. $\times 428$.

region occupied by the cicatrice there has been division and multiplication of the cells of both the palisade and spongy parenchyma which has resulted in a band of tissue without intercellular spaces. The wound periderm is several cells in width. The phellem is made up of a mass of large cells whose thick walls are not only suberized but lignified as well. The cells lying nearest the lesion are filled with a dense granular substance resembling tannin, while the remaining cells are apparently devoid of contents. The epidermal cells of this layer also have suberized walls. Both a phellogen and phelloderm are present in the wound periderm. The cells comprising these two layers are thick-walled but this thickening is entirely cellulose in nature. Chloroplasts are absent from these cells. The phellogen consists of a single layer of cells which are filled with dense protoplasm, while the cells of the phelloderm contain only a peripheral layer. Nuclei are plainly visible in the cells of both of these layers. The inner part of the cicatrice is com-

posed of somewhat enlarged, thin-walled living cells, with only a few chloroplasts present.

All efforts failed to demonstrate the presence of the pathogene beyond the edge of the cicatrice.

The reaction to the leaf to wounding (Fig. 2) is not unlike that induced

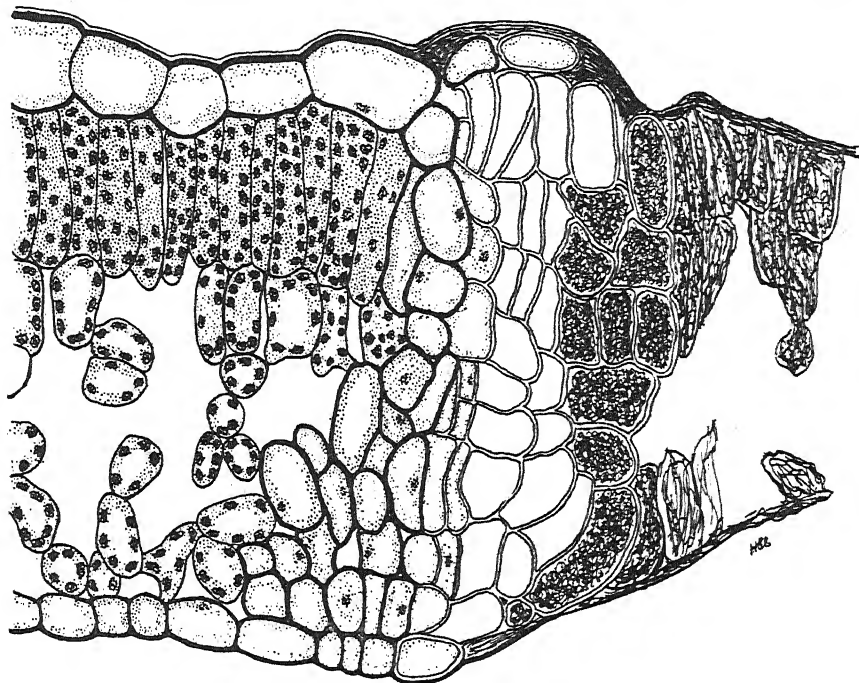


FIG. 2. Cross section through the edge of a wound on a leaf of *Prunus domestica* showing the cicatrice formed under these conditions. $\times 428$.

by the pathogene in question. There are, however, certain differences which seem to be characteristic. The principal difference appears in the cells of the phellem layer. In the case of wounding, the cells are somewhat smaller and the radial arrangement is more definite. The leaf in the region of the cicatrice is usually somewhat thicker than normal. The cicatrice is formed at some little distance from the edge of the wound. At the edge of the wound the cells have entirely collapsed and this condition becomes less evident as we approach the cicatrice. Many of these collapsed cells, both in the palisade and in the spongy parenchyma, are more or less filled with the tannin-like substance already referred to.

Lesions produced by Coccomyces lutescens Higgins on Prunus virginiana L. The structure of the healthy leaf of *Prunus virginiana* is very similar to that of *Prunus domestica*. The leaf of the former species, however,

is somewhat thinner, and at intervals resin cavities are formed in the upper part of the palisade parenchyma.

The lesions on this suscept are also very similar in appearance to those on *Prunus domestica* and need no further description.

When viewed in cross-section the type of cicatrice is seen to differ in some respects from that described on *Prunus domestica*. At the edge next the lesion the leaf is fully one-third thicker than normal and gradually decreases in thickness to the point where the cicatrice merges into healthy leaf tissue. Throughout the whole cicatrice there has been a multiplication and division of the cells of the spongy parenchyma and also of the lower palisade cells which has resulted in the occlusion of the intercellular spaces. The wound periderm is of considerable width. The phellem layer, which is several cells wide, is made up of much enlarged cells, more or less irregular in shape, whose walls are lignified as well as suberized. While they are not so regular in their arrangement as are the phellem cells in the wound periderm in *Prunus domestica*, they evidently have their origin in the phellogen. The epidermal cells in this layer are slightly enlarged and their walls have also become suberized. The remainder of the wound periderm is made up of thick-walled cells. The outer one or two rows lying next the phellem constitute the phellogen and are filled with protoplasm while the remaining rows contain only a peripheral layer. No chloroplasts are present in these cells. The inner layers of the cicatrice is made up of thin-walled living cells. Those nearest the wound periderm contain no chloroplasts while the number of chloroplasts is relatively few in the cells adjacent to the normal tissue. Dead cells, remnants of the diseased area, may or may not be found clinging to the outer edge of the phellem. No trace of the pathogene could be found beyond the cicatrice.

The cicatrice which is formed as a result of wounding is so similar to that formed on *Prunus domestica* under similar conditions that little further description is necessary. The chief difference lies in the fact that it is not so extensive, there being at most only two layers of cells in the phellem and these very much enlarged.

Lesions produced by Cocomyces hiemalis Higgins on Prunus cerasus L. With certain minor differences the general structure of the leaf of *Prunus cerasus*, as studied in cross section, is similar to that of the *Prunus sp.* already described. The cells of both the lower and upper epidermis are irregular in size and shape. While some of the upper epidermal cells contain the usual thin layer of protoplasm, many of them are filled with a dense substance which stains deeply with haemotoxylin. An occasional cell of the lower epidermis is also filled with this same substance.

The type of lesion found on this suscept is very similar to that described on *Prunus domestica*. A study of a cross-section made through one of these

lesions shows a cicatrice formed at the margin which is almost identical with that described on *Prunus domestica*. Here we find the same phellem layer of enlarged cells having suberized and lignified walls and apparently lacking in cell contents. Between this layer and the normal tissue there is a layer of thick-walled living cells.

Wounding of the leaf of this susceptible results in the formation of a cicatrice so similar in its general characters to that formed on *Prunus domestica* under the same conditions that no detailed description need be given.

Lesions produced by Cocomyces hiemalis Higgins on Prunus avium L. The structure of the healthy leaf of this susceptible is essentially the same as that of *Prunus cerasus* and the type of lesion produced by the pathogene is the same as that produced by the other species of *Cocomyces* on the susceptibles studied.

As in the case of the other species of *Prunus* dealt with in this paper, there is a definite cicatrice formed at the edge of the lesion. A study of a cross-section through this formation shows that in its general structure it is similar to that already described on *Prunus domestica*.

Wounded leaves formed a cicatrice in all respects like that formed on the other species of *Prunus* when wounding was practiced.

Lesions produced by Mycosphaerella sentina (Fr.) Schr. on Pyrus communis L. In the healthy leaf of *Pyrus communis* the lower epidermis is very much like the upper in its general characters. The outer cell walls of the latter, however, are somewhat thicker than are the outer cell walls of the former. In both cases occasional cells are partly filled with a dense staining material. The palisade parenchyma consists of two layers of closely packed cells, those of the upper layer being much longer than those of the lower. The cells of the spongy parenchyma are variable as to size and shape and are loosely arranged.

The lesions on the leaf are oval to irregular in outline, are entirely holonecrotic in character, and are brown in color with a grayish-white center. The margin is somewhat darker in color than is the main body of the lesion; is slightly raised and sharply defined.

At the edge of the holonecrotic area there is a very definite cicatrice laid down. When viewed in cross-section this is seen to be similar to that described on *Prunus domestica*. The outer edge of the cicatrice ends abruptly in normal tissue, and by none of the methods used was it possible to find any evidence of the pathogene beyond this barrier.

Cross-sections of leaves artificially wounded reveal the formation of a cicatrice at some little distance from the edge of the wound. The intervening cells are dead and in some cases the epidermis has apparently folded over the edge of the wound. In all respects this cicatrice is similar to that formed at the edge of the lesion caused by the pathogene.

Lesions produced by Cercospora beticola Sacc. on Beta vulgaris L. The healthy leaf of *Beta vulgaris* as seen in cross-section is rather open in structure. The upper epidermis is composed of cells which are more or less irregular in outline. The outer and radial walls are comparatively straight but the inner walls tend to curve inward, at times even forming an obtuse angle. The outer walls are considerably thickened, the radial walls somewhat less so, while the inner walls are only slightly thickened. The cells of the lower epidermis are somewhat different from those of the upper, being slightly longer, narrower, and with the inner wall more parallel to the outer. Both the outer and radial walls are noticeably thickened. The palisade parenchyma is very open in structure. Large intercellular spaces are pres-

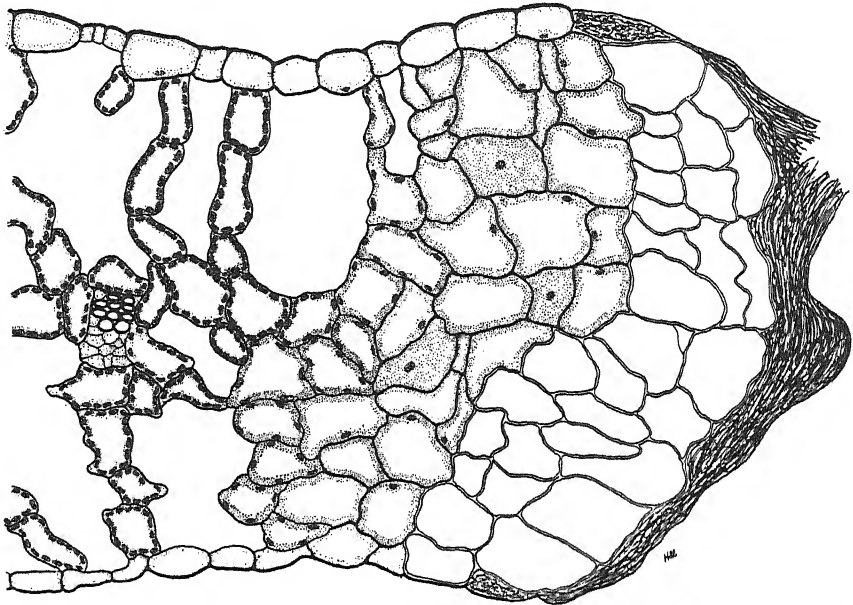


FIG. 3. Cross section through the edge of a lesion on a leaf of *Beta vulgaris* caused by *Cercospora beticola*. $\times 225$.

ent, and in cross section of the leaf the cells appear in single rows, or at most in groups of two or three, each row comprising two or three elongated cells placed end to end. In the material sectioned no large veins were present, but in the presence of small veins the structure of the palisade parenchyma was unaltered. The spongy parenchyma is also very open in structure. While the cells are a bit more irregular in shape and arrangement, this tissue does not differ essentially from the palisade parenchyma.

The infected areas on the leaves appear as spots of varying size, more or less regular in outline, holonecrotic in character, the center of which is sunken and grayish-white in color, surrounded by a purplish-red margin.

A cross-section of the leaf made through one of these lesions (Fig. 3) shows that there has been a marked change in the character of the tissue adjacent to the sunken area. For some distance back from this region there has evidently been renewed meristematic activity in the cells of the mesophyll which has resulted in complete occlusion of the intercellular spaces and the formation of a definite cicatrice. In general the region occupied by the cicatrice is somewhat thinner than that of the healthy portion of the leaf, although there was considerable variation in this respect in the material examined. The variation may possibly have been due

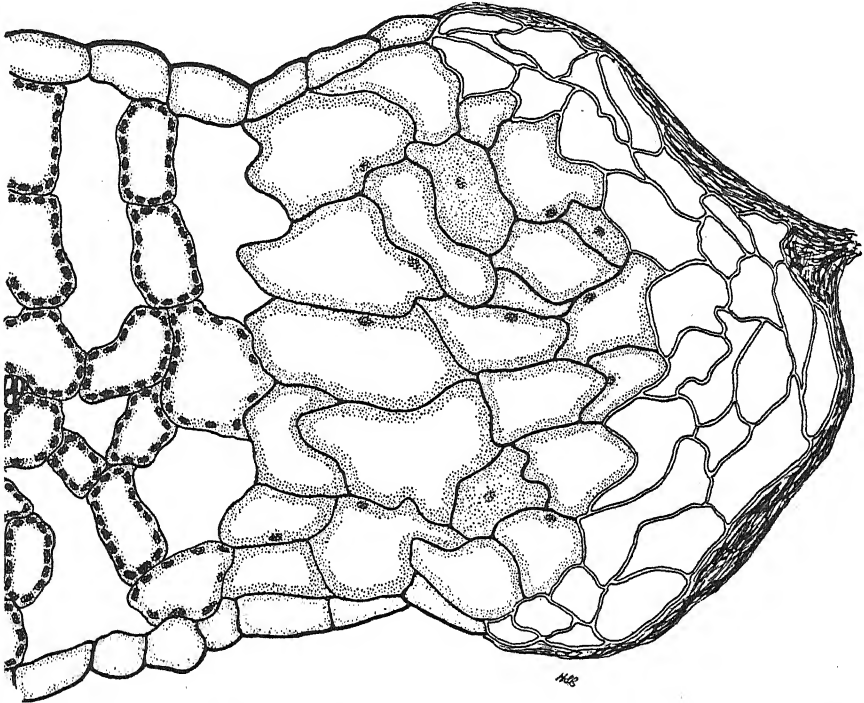


FIG. 4. Cross section through the edge of a wound on a leaf of *Beta vulgaris* showing the cicatrice formed under these conditions. $\times 300$.

to differences in the maturity of the wounded leaves. The cicatrice does not differ essentially in character from those already described on species of *Prunus*. The cells, however, are larger and more irregular in shape, while the thickening of the walls is not so pronounced. There is a mass of dead collapsed cells attached to the outer edge of the phellem layer. All efforts failed to demonstrate the presence of the fungus either within or behind the cicatrice.

The cicatrice formed as a result of wounding (Fig. 4) is very similar in its general characters to that just described. The cells of the phelloderm

as shown in the illustration are very large but there is such a variation in this respect in the different sections that one cannot consider this a constant character of wounded leaves.

Discussion

The evidence presented in the foregoing pages shows that the species of *Prunus*, *Pyrus*, and *Beta* studied may be expected to react, when attacked by necrogenic pathogenes, by the formation of a cicatrice which isolates the infected portion from the healthy tissue. The character of this cicatrice and the extent of the healing tissue differs with the relation of the infected portion to veins and islet borders. Undoubtedly there is also variation with the age of the leaf at the time at which infection occurs. It is also possible, in fact quite probable, that environmental factors play an important part not only in the character of the cicatrice formed but in the initiation of the process as well.

The character of the cicatrice, with certain minor differences, is essentially the same in all of the species studied. The most striking features of this structure are the renewal of the meristematic condition in the mature mesophyll cells which results in a multiplication of cells in this region, the occlusion of intercellular spaces, and the formation of a definite wound periderm with its phellogen layer which is active in the production of cork cells. These cork cells are frequently greatly enlarged. This enlargement of a layer of cells about the "shot-hole" in leaves of *Prunus sp.* is pointed out by Higgins (7), although he makes no reference to their origin from a cork cambium. That such a cambium may be formed in diseased leaves of *Prunus amygdalus* has been clearly shown by Samuel (17), and a similar condition is reported by McWhorter (12) on diseased leaves of *Carica papaya*.

The experiments with wounded leaves indicate that in the species studied the natural reaction of the susceptible to injury is the formation of a cicatrice. Such a cicatrice is similar in its general characters to that formed at the edge of necrotic lesions caused by pathogenic organisms. That the formation of a cicatrice of some nature, as a result of mechanical injury, is of common occurrence in the leaves of many higher plants is evident from the work of Blackman and Matthaei (1), Wyncken (24), Wylie (23), Woit (20), and others.

In the present work no attempt was made to study the development of the healing tissue nor the factors influencing its formation. The work of Samuel (17) is interesting in this connection and deals in considerable detail with the development of this healing tissue in *Prunus amygdalus*, while the work of Blackman and Matthaei (1) is of equal interest in connection

with the formation of healing tissues in leaves subjected to traumatic stimulation.

The fact that in no case where a typical wound periderm is formed about the edge of the lesion was it possible to find any evidence of the hyphae beyond this point would indicate that this periderm was an effectual barrier against the advance of the fungus. This is not conclusive proof, however, as certain physiological factors may be involved and, furthermore, McWhorter (12) has shown that this layer may be penetrated to a slight extent by *Cladosporium papayae*.

*Diseases in which no Definite Cicatrice is Formed
about the Edge of the Lesion*

By far the larger number of the diseases studied were found to fall in this group. The species of pathogenes involved belong to several genera and occur upon a wide variety of susceptibles among the flowering plants.

As there seems to be no correlation either between the species of susceptible or the species of pathogene and the reaction of the susceptible to the advance of the organism, the observations have been grouped according to the genus to which the pathogene belongs.

Lesions produced by Septoria petroselini Desm. on *Apium graveolens* L. Both the upper and the lower epidermis of the leaf of *Apium graveolens* are of medium thickness and are made up of rather large cells. The palisade parenchyma consists of a single row of rather short broad cells somewhat openly arranged. The spongy parenchyma is made up of cells which vary greatly in both shape and size, some being comparatively small and more or less globose in outline, while others are large and elongated.

The lesions caused by the fungus are small but often coalesce to form large, irregular areas. The main body of the lesion is holonecrotic in character and brownish-gray in color. This holonecrotic area is surrounded by a yellowish-green plesionecrotic zone which is variable in width.

A study of a cross-section through one of the older lesions (Fig. 5) reveals the fact that the cell contents have entirely disappeared and the walls have collapsed. The cell walls are brown, and there is an abundance of intercellular mycelium. The fungous hyphae are plainly visible in the plesionecrotic area and extend to the margin of normal leaf tissue. Those cells in the region of the tips of the advancing hyphae are apparently unchanged but in the intervening cells the contents are broken down and this condition is more complete nearer the necrotic area. In no case is there any evidence of cicatrice formation.

Lesions produced by Septoria podophyllina Pk. on *Podophyllum peltatum* L. In the healthy leaf of *Podophyllum peltatum* both the upper and

lower epidermis are composed of rather large, irregular, thin-walled cells. There is no well-defined palisade parenchyma but there is a layer of irregularly shaped, somewhat loosely arranged cells, lying just under the upper epidermis. The spongy parenchyma is open, particularly in the lower portion where the globose to elongate cells are arranged in chains, or groups, to enclose large air spaces.

On the material collected the lesions vary greatly in size and are more or less irregular to angular in outline. In many cases this irregularity in outline is due to veins at the edge of the lesion. The lesion is composed of a brown holonecrotic area surrounded by a yellowish-green plesionecrotic zone of varying width. This may be sharply delimited from the normal green tissue of the leaf or may blend gradually into it.

In the necrotic area the cell contents have disappeared and the walls have collapsed. The intercellular hyphae are abundant in this region.

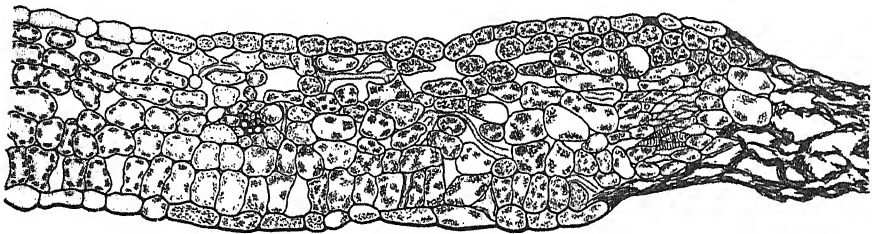


FIG. 5. Cross section through a diseased leaf of *Apium graveolens* showing the histologic changes induced by *Septoria petroselinii*. $\times 171$.

The hyphae extend through the plesionecrotic zone and at the outside limit of growth apparently lie between normal cells.

Artificial wounds were made in leaves of *Podophyllum peltatum*. There is no formation of a wound periderm such as is found in *Prunus* sp. but there are certain changes in the character of the contents and in the walls of those cells adjoining the wound. For some distance back from the point of wounding all of the leaf tissues are dead and collapsed, and these collapsed cells contain a brownish substance resembling tannin. Adjoining the inner edge of this collapsed area, and lying between it and normal tissue, there are a few cells which are filled with this brownish substance and whose walls give a positive reaction for suberin. In some cases these cells also may be partly collapsed. There is apparently no increase in either number or size of cells in this region.

Lesions produced by Septoria conspicua Ell. et Mart. on *Steironema ciliatum* (L.) Raf. The structure of the healthy leaf of *Steironema ciliatum* is rather open. The leaf is very thin but the upper epidermis and also the

lower are quite thick as compared with the total thickness of the leaf. Both epidermal layers are made up of large cells. The palisade parenchyma consists of a single layer of short, broad cells rather loosely arranged. The cells of the spongy parenchyma vary considerably both in size and shape.

On the leaves of this suscept the lesions are circular in outline and rather uniform in size. The central portion of the holonecrotic area is dark brown in color while the margin is a very dark brown. This area is surrounded by a pale yellowish plesionecrotic zone.

The diseased portion is very little thinner than is the normal leaf. In the holonecrotic area the epidermal cells have only partially collapsed. Many of the palisade cells in this region, as well as some of the cells of the spongy parenchyma, are completely filled with a dense granular substance which did not react to any of the stains used. These cells are only slightly shrunk, and in many parts of this area the intercellular spaces are completely filled with the large hyphae. In the plesionecrotic zone the cells of the mesophyll are filled with the same dense granular substance, and occasional intercellular strands of hyphae can be found. Beyond these granulated cells the tissue is normal. There is no other apparent change in the character of the cells in this region.

Lesions produced by Septoria cirsii Niessl. on Cirsium arvense (L.) Scop. The leaf of *Cirsium arvense* is open in structure. The epidermal cells are large and comparatively thick-walled. There is no well-defined palisade parenchyma but there is a layer of loosely arranged, irregular shaped cells lying just beneath the upper epidermis. The remainder of the mesophyll tissue is quite open, the cells varying greatly in both size and shape and arranged more or less in rows, or groups, enclosing large air spaces.

The lesions on the leaves are somewhat irregular in size and shape. The main portion of the lesion is holonecrotic in character, brownish in color, with a well-defined margin. This is surrounded by a narrow, greenish-yellow, plesionecrotic zone. These lesions frequently coalesce to form large irregular patches.

In the holonecrotic area the cell contents have completely disappeared, the cells have collapsed, and the leaf is much thinner at this point. Threads of hyphae are visible between these cells and also in the intercellular spaces of the plesionecrotic zone. The cells comprising this zone have their contents largely disorganized and the walls are partially collapsed. In no case was there any evidence of cicatrice formation.

Lesions produced by Septoria verbasicola B. et C. on Verbascum blattaria L. The healthy leaf of *Verbascum blattaria* is rather thick and in cross-section the cells of the upper epidermis are seen to be much larger

than those of the lower. In both cases the inner and outer walls are thickened. The palisade parenchyma is made up of two or three layers of rather short, broad cells. Occasionally several of the cells of the upper layer have broken down to form a large cavity. The cells of the spongy parenchyma are somewhat irregular in shape and rather compactly arranged.

The lesions in this suscept are variable in size, more or less oval in outline, and frequently coalesce. The holonecrotic center is grayish-white in color while the plesionecrotic area surrounding it is purplish-red and tends to blend into the normal green of the leaf.

In the holonecrotic area the cell contents have entirely disappeared and the walls are completely collapsed. The hyphae are abundant between these collapsed cells, can be seen in the intercellular spaces in the plesionecrotic area, and extend to the edge of normal tissue. The nuclei and chloroplasts have disappeared from most of the cells in this area, some being entirely empty although they still retain their shape. There is no multiplication of cells nor change in the character of the cell walls about the margin of the lesion.

Lesions produced by Septoria acerina Pk. on *Acer pennsylvanicum* L. The healthy leaf of *Acer pennsylvanicum* is comparatively thin and many of the cells of both the upper and lower epidermis are filled with a dense tannin-like substance. The palisade parenchyma is made up of a single layer of much elongated cells which are closely packed together. The spongy parenchyma is rather open in structure and is composed of cells which vary greatly in size and shape.

The lesions caused by this fungus are small, irregular in outline, and frequently coalesce to form larger areas. The holonecrotic center is dark brown in color and is surrounded by a broad, light-colored, clearly defined plesionecrotic zone.

With an occasional exception, the contents have entirely disappeared from the cells in the necrotic area. The epidermal cells and the cells of the spongy parenchyma have collapsed. Many of the palisade cells have also collapsed and those that have not are very much shortened. The intercellular hyphae are abundant throughout the whole of this area and extend through the plesionecrotic zone to the edge of normal tissue. The contents of the cells in this region are somewhat disorganized but apparently no shrinkage has taken place. Occasional palisade cells, or even groups of two or three of these cells, have densely granular contents. In no case was there any indication of a change in the character of the cell walls except that common to the necrotic area.

Lesions produced by Septoria osmorhizae Pk. on *Osmorhiza longistylis* (Torr.) D. C. The leaf of *Osmorhiza longistylis* is very thin. The upper

epidermal cells are large and oval to elongate in shape, while those of the lower epidermis are uniformly narrow and much elongated. There is no well-defined palisade parenchyma. The cells of the spongy parenchyma vary from oval to elongate in shape and are arranged parallel to the epidermis.

The lesions on the leaf are small and ovate to angular in outline. The central holonecrotic portion is grayish-white in color while the plesionecrotic zone surrounding it is somewhat darker.

The intercellular hyphae are abundant in the holonecrotic area. The cell contents in this portion of the leaf have entirely disappeared and the cell walls have collapsed, resulting in a tangled mass of cell walls and hyphae. Where there are no large veins the hyphae can be traced for some distance into the plesionecrotic zone and the tips of the advancing hyphae are not more than two or three cells removed from normal tissue. The cells of this region are in various stages of disorganization, depending on the distance they are removed from the holonecrotic area. In no case was there any evidence of cicatrice formation.

Lesions produced by Cercospora caulophylli Pk. on Caulophyllum thalictroides (L.) Michx. The leaf of this suscept is very thin. The upper epidermal cells vary greatly in size and shape, some being very long with the outer and inner walls almost parallel, while others are oval to almost globose. The outer walls are slightly thickened. Except that the cells are smaller and somewhat thinner-walled, the lower epidermis is similar in its structure. The palisade parenchyma consists of a single layer of short, rather broad cells, more or less rectangular in outline. The entire contents of these cells stain deeply with haemotoxylin. The spongy parenchyma cells are globose to elongate in shape and are loosely arranged in such a manner as to enclose large air spaces.

The lesions in these leaves vary in size and are somewhat angular and irregular in outline. The holonecrotic area is dark brown in color, although in many of the larger lesions this may be a lighter brown in the center. The margin is clear-cut and is surrounded by a yellowish-green plesionecrotic zone of varying width. In some cases the lesions may coalesce to form large, irregular-shaped areas.

A study of a cross-section through one of these lesions shows that in the holonecrotic area the epidermal cells and the cells of the spongy parenchyma have completely collapsed while in the cells of the palisade parenchyma this collapsed condition is only partial. In the plesionecrotic zone the cell contents are more or less disorganized but the walls have not collapsed. In this region the intercellular hyphae can be traced to within two or three cells of normal tissue. Apparently there is no histologic reaction which would in any way hinder the advance of the pathogene.

Lesions produced by Cercospora symlocarpi Pk. on *Symlocarpus foetidus* (L.) Nutt. The upper epidermal cells of *Symlocarpus foetidus* are large and for the most part much longer than broad, with the outer walls fairly straight and the inner ones curving strongly downwards. Both the outer and radial walls are slightly thickened. The lower epidermis is similar to the upper except that the walls are scarcely thickened. The palisade layer is open in structure and consists of a single layer of very short, broad, rectangular cells. Just beneath the palisade layer and making up a part of the spongy parenchyma are two or three rows of cells varying in size and shape but placed for the most part with their long axes parallel to the epidermis. The remainder of the spongy parenchyma is made up of chains of thin-walled cells, irregular in shape and size, and arranged in such a manner as to enclose large air spaces. These cells contain relatively few chloroplasts.

The lesions on the leaf are large, oval in shape, and irregular in outline save where they are bounded by veins. The holonecrotic center is brown in color, shading into the dark brown or black plesionecrotic border. This border is often somewhat raised and is sharply outlined.

A study of a cross-section through one of these lesions (Fig. 6) shows a general collapse of the cells in the holonecrotic area. The plesionecrotic zone is very narrow and consists at most of three or four cells. The nuclei and chloroplasts have largely disappeared from these cells but apparently there has been no shrinking of the walls. In some cases the upper epidermal cells for some distance beyond the necrotic area have collapsed but the cells beneath are apparently normal. No trace of the hyphae was found beyond the holonecrotic region.

In the case of artificial wounding, the cells a short distance back from the wound have entirely collapsed. At the inner edge of this area there is a slight enlargement of the cells of the palisade parenchyma and of those cells lying just beneath it. The walls of these enlarged cells have become suberized, as have also the walls of the epidermal cells bordering upon this area.

Lesions produced by Cercospora menispermii Ell. et Hollw. on *Menispermum canadense* L. The leaf of *Menispermum canadense* has a heavy upper epidermis composed of large cells with much thickened outer walls. The lower epidermis is also made up of large cells but the outer walls are relatively thin. The palisade parenchyma is made up of a single layer of closely packed, elongated cells and a second layer which is more loosely arranged. The spongy parenchyma is very open with large irregular air spaces.

The brownish lesions on the leaf are somewhat irregular in shape and

size and are largely holonecrotic in character. The narrow plesionecrotic margin is purplish-black in color and well-defined.

In the holonecrotic area the leaf is somewhat thinner than normal. This is due to the collapse of the epidermal cells and those of the spongy parenchyma. The cells of the palisade parenchyma are much reduced in length and are either empty or partially filled with a brownish substance. The intercellular hyphae are abundant in this region but apparently do not extend into the plesionecrotic zone. There is no change in the character of the cells at the edge of the lesion indicating the formation of a cicatrice.

✓*Lesions produced by Cercospora circumcissa* Sacc. on *Prunus serotina* Ehrh. The upper epidermis of *Prunus serotina* is somewhat thicker than is the lower but otherwise they are very similar. Both the outer and inner walls of these cells are considerably thickened. The palisade parenchyma

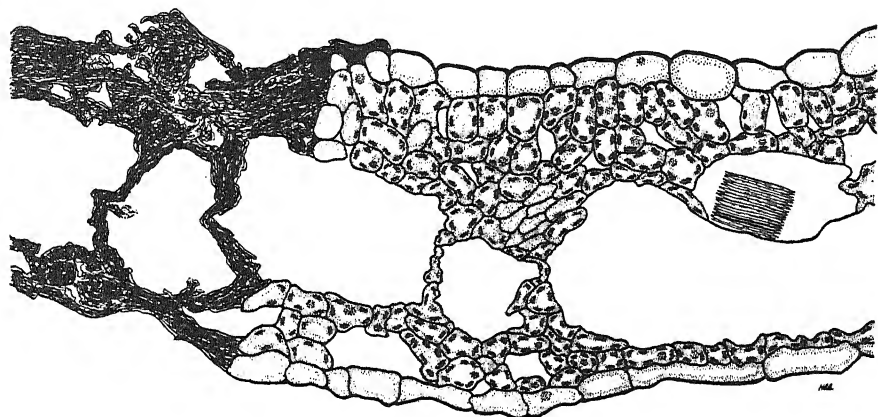


FIG. 6. Cross section through a diseased leaf of *Symplocarpus foetidus* showing the histologic changes induced by *Cercospora symplocarpi*. $\times 197$.

consists of a single layer of cells which are somewhat elongated and taper slightly toward the lower end. These cells are rather compactly arranged, although there are evident intercellular spaces between many of them. The cells of the spongy parenchyma are irregular in size and shape and are so arranged that they enclose large air spaces.

Lesions produced on this susceptible are angular in outline and quite variable in size. The holonecrotic portion varies in color from a light brown to a gray and is bordered by a reddish-brown plesionecrotic zone.

In the holonecrotic area the leaf is somewhat thinner than normal. The contents of the cells of the spongy parenchyma have disappeared and the walls have collapsed. This is also the case in many of the palisade cells, but some are partially filled with a dense granular substance. The hyphae are not at all plentiful in any part of the lesion, but occasional threads are

visible between the cells in both the holonecrotic and the plesioneecrotic areas. The contents of the cells of the latter region are more or less disorganized. There was no evidence of cicatrice formation at the edge of the lesion.

Lesions produced by Ramularia primulae Thüm. on Primula polyantha Mill. The upper epidermis of *Primula polyantha* is much thicker than is the lower. It is made up of much larger cells, the outer walls of which are markedly thickened. Occasional cells in both of these layers are filled with a dense, tannin-like substance. The palisade parenchyma is made up of a single layer of short thick cells, loosely arranged. The spongy parenchyma is composed of cells of various sizes and shapes and is moderately open in arrangement.

The lesions on the leaf of this suscept are variable in size and somewhat irregular in outline. The holonecrotic center is grayish in color with a sharply defined somewhat darker margin. Surrounding this area there is a rather broad yellowish-green plesioneecrotic zone. The lesions are numerous upon the leaf and often unite to form large necrotic patches.

In the holonecrotic area the cell contents have broken down and disappeared. The cell walls have collapsed to a large extent, leaving the leaf somewhat thinner than normal at this point. In the outer edge of this region the epidermal cells are collapsed (Fig. 7) and the contents of the mesophyll cells are in various stages of disorganization. The intercellular hyphae, although not abundant in the holonecrotic area, are plainly visible and extend through the plesioneecrotic zone to a point within a few cells of normal tissue. In the plesioneecrotic zone none of the cells are collapsed. In the mesophyll cells of this region the nuclei and chloroplasts have disappeared and the cytoplasm is partially broken down. There is no evidence of any healing tissue at or near the edge of the lesion which would tend to prevent the advance of the pathogene.

Lesions produced by Ramularia ranunculi Pk. on Ranunculus recurvatus Poir. The leaf of *Ranunculus recurvatus* is extremely open in structure. The upper and lower epidermis are very similar in structure and are composed of rather irregularly shaped cells whose outer walls are slightly thickened. There is no well-defined palisade parenchyma, but the irregularly shaped cells which compose the mesophyll are more closely arranged in that region.

The lesions on the leaves of this suscept are large, circular to oval in outline except where they are bounded by large veins. They are entirely holonecrotic in character and brownish in color with somewhat darker centers.

In the center of the diseased area the contents of all of the cells have disappeared and the walls have collapsed, thus causing the leaf to be much

thinner than normal at this point. Farther out toward the edge of the lesion the epidermal cells may or may not be collapsed and the leaf gradually increases to normal thickness although the contents of the mesophyll cells may be entirely disorganized and the walls partially shrunken. The threads of hyphae can be seen in the intercellular spaces of the mesophyll and extend to the very edge of normal tissue. There is no evidence of any susceptible reaction of any kind which would tend to prevent the advance of the pathogene.

Lesions produced by Ramularia decipiens Ell. et Ev. on Rumex crispus L. The leaf of *Rumex crispus* is very open in structure. The cells of the upper epidermis contain a dense granular substance in the protoplasmic layer, and in some cases the cells are completely filled with this material. The palisade parenchyma is composed of a single layer of rather short, broad

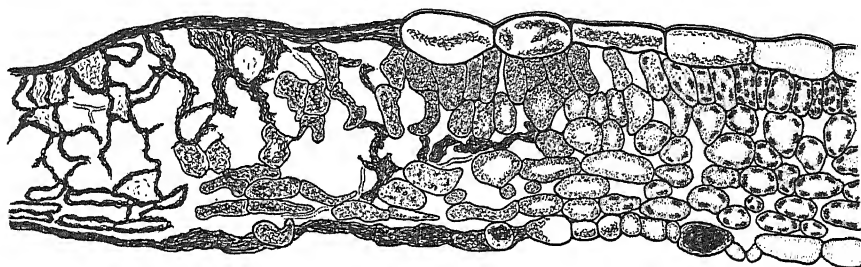


FIG. 7. Cross section through a diseased leaf of *Primula polyantha* showing the histologic changes induced by *Ramularia primulae*. $\times 187$.

cells, loosely arranged. The cells of the spongy parenchyma vary widely in size and shape.

The lesions vary greatly in size and are more or less irregular in shape. The central part of the holonecrotic area is light brown in color with a wide, somewhat darker-colored margin. This is usually surrounded by a purplish plesionecrotic zone.

The holonecrotic area is very little thinner than normal. The cell contents in this part of the leaf have entirely disappeared. The epidermal cells, and to a certain extent the cells of the spongy parenchyma, have collapsed. The hyphae are intercellular and can be traced throughout the plesionecrotic zone to the very edge of normal tissue. In this zone the cells of the epidermis and the mesophyll are in various stages of disorganization and collapse. There is no evidence of histologic changes which would tend to prevent the advance of the pathogene.

Lesions produced by Alternaria solani (E. et M.) Jones et Grout on Solanum tuberosum L. The structure of the healthy leaf of *Solanum tuberosum* is rather open. The upper epidermis is much thicker than the

lower and the cells are more or less filled with some granular substance. The outer walls of the cells of both the upper and lower epidermis are slightly thickened. The cells of the palisade parenchyma are narrow, much elongated, and irregular in outline with large air spaces between the individual cells. The cells of the spongy parenchyma are irregular in shape, and the air spaces in this tissue are also large.

The lesions on this suspect are entirely holonecrotic in character, irregular in shape and size, somewhat angular in outline and marked with concentric rings. The color is dark brown and the margin is well defined.

In the diseased area the upper epidermal and palisade cells are filled with a dense granular substance (Fig. 8). The cells of the spongy paren-

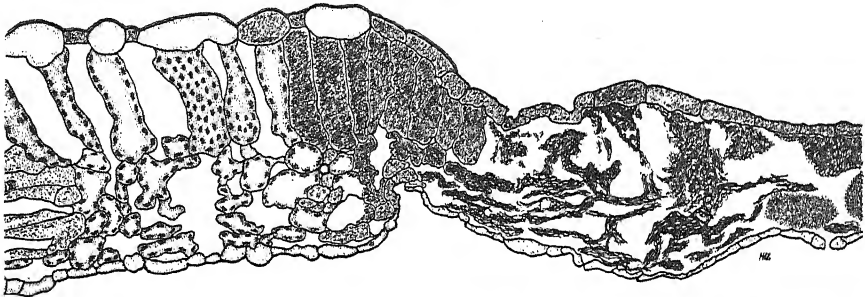


FIG. 8. Cross section through a diseased leaf of *Solanum tuberosum* showing the histologic changes induced by *Alternaria solani*. $\times 145$.

chyma are collapsed, as are also the cells of the lower epidermis. The palisade cells are much reduced in length and partially collapsed except in the region of the concentric rings where they stand out more prominently. The necrotic area ends almost abruptly in normal tissue, although the cells on the outer edge have not yet collapsed. The hyphae are abundant between the shrunk mesophyll cells, particularly in the spongy parenchyma, and can be traced almost to the edge of the normal tissue. There is no evidence of cicatrice formation.

Lesions produced by Alternaria solani (E. et M.) Jones et Grouet on Lycopersicum esculentum Mill. The general structure of the leaf of *Lycopersicum esculentum*, the appearance of the lesions, and the reaction of the suspect to the invasion of the pathogene are almost identical with those just described for *Solanum tuberosum*.

Lesions produced by Macrosporium cucumerinum Ell. et Ev. on Cucumis melo L. The leaf of this suspect is quite thick. Both the lower and upper epidermal cells are large and their outer walls are slightly thickened. The palisade parenchyma is made up of a single layer of narrow, much

elongated cells, rather closely arranged. The cells of the spongy parenchyma are variable in shape and size and are arranged somewhat loosely.

The lesions caused by this pathogene are variable in size, regular in outline except where they are bounded by veins, and frequently coalesce to form large necrotic areas. The holonecrotic center is light brown in color. A narrow plesionecrotic zone surrounds this area but is more marked on the under surface of the leaf than on the upper.

In the holonecrotic area the leaf is much thinner than normal, the cell contents have disappeared, and the walls have collapsed. This collapsing of the walls is more complete in the cells of the spongy parenchyma. These shrunken cells are interwoven with the intercellular hyphae and they are present in the plesionecrotic zone, extending to a distance of one or two cells from normal tissue. The contents of the cells in this zone are partially broken down but the walls have not collapsed.

Lesions produced by Phyllosticta fraxinii Ell. et Mart. on Fraxinus americana L. In the leaves of *Fraxinus americana* the upper and lower epidermis are quite similar in structure except that in the former the cells are filled with some dense granular substance. The palisade parenchyma is made up of a double layer of narrow, elongated cells which are arranged to form a compact wall. The cells of the spongy parenchyma vary in size and shape and are rather closely arranged, the intercellular spaces being comparatively small.

The lesions on this leaf are large and more or less irregular in outline. The main body of the lesion is holonecrotic in character and light brown in color. It is surrounded by a purplish-black plesionecrotic zone of varying width.

In the holonecrotic area the epidermal cells and the cells of the spongy parenchyma are empty and collapsed. The palisade cells are slightly reduced in length, somewhat shrunken, and partially filled with some dense granular material. The leaf at this point, however, is not much thinner than normal. The contents of the cells in the plesionecrotic zone are somewhat disorganized. The hyphae are visible between the cells in this region and apparently advance to within two or three cells of healthy tissue.

Lesions produced by Pseudopeziza medicaginis (Lib.) Sacc. on Medicago sativa L. Both the upper and lower epidermis of *Medicago sativa* are similar in structure, the cells varying considerably in size and shape, with the outer walls markedly thickened. The palisade cells are arranged in one or two layers, some being rather short and broad while others are much elongated. They are somewhat open in arrangement. The cells of the spongy parenchyma are also loosely arranged and vary greatly both in size and shape.

The lesions are numerous and scattered on the leaves. They appear as small holonecrotic areas which are dark purple in color and surrounded by a yellowish plesionecrotic border which blends into the normal green of the leaf.

In the holonecrotic area the palisade cells are almost entirely replaced by the stroma of the fruiting body while the cells of the spongy parenchyma are either collapsed or filled with the hyphae. In the plesionecrotic zone the epidermal cells may be collapsed. The hyphae can frequently be seen in one or more cells beyond this point and occasional threads of hyphae can be seen in the mesophyll cells of this region and may extend to within two or three cells of normal tissue. The cells in this zone have their contents more or less disorganized and an occasional palisade cell is filled with some dense brownish substance, but there is little if any collapsing of the cell walls. There is no evidence of cicatrice formation.

Lesions produced by Pseudopeziza medicaginis (Lib.) Sacc. on Medicago lupulina L. The structure of the leaf of *Medicago lupulina* differs from that of *Medicago sativa* in being very much thinner. This difference in thickness is mainly due to the shorter palisade cells but is also due to some extent to a thinner layer of spongy parenchyma.

The lesions on this suscepr are very similar in their general characters to those described on *Medicago sativa*. The suscept reactions and the extent of the mycelial invasion are in all respects similar to those just described for *Medicago sativa*.

Lesions produced by Pseudopeziza trifolii (Bernh.) Fekl. on Trifolium pratense L. The leaf of *Trifolium pratense* resembles that of *Medicago sativa* in its general structure. The palisade cells are not so long, however, and although the leaf blade is approximately the same thickness in the two species, the lack in length of palisade cells is made up by a corresponding increase in thickness of spongy parenchyma in *Trifolium pratense*.

In general the character of the lesions on the leaves is similar to that given for the lesions on leaves of *Medicago sativa*. The suscept reactions and the extent to which the hyphae have penetrated the leaf tissues are the same as have been described in connection with *Medicago sativa*.

Lesions produced by Pseudopeziza ribis Kleb. on Ribes grossularia L. The leaf of *Ribes grossularia* is rather thick. The upper epidermis differs from the lower in having larger cells whose walls are somewhat thicker. The palisade parenchyma consists of two or even three layers of cells. These cells are quite broad and those of the upper layer are much longer than those of the lower. They are closely packed together. The cells of the spongy parenchyma are variable in size and shape. They are arranged in chains or groups and, while only moderately open in arrangement, the intercellular spaces are at times quite large.

The lesions on this suscept are small, more or less round in outline with an indefinite margin. The color is a dark purple with a grayish center in the older lesions.

The condition existing here as a result of the invasion of the pathogene is so similar to that already described for *Pseudopeziza medicaginis* that no further description is necessary.

Lesions produced by Rhytisma ilices-canadensis Schw. on *Ilex verticillata* (L.) Gray. A study of the leaf of *Ilex verticillata* shows the upper epidermal cells to be very regular in shape and size, almost rectangular in outline, and with a heavy outer wall. The lower epidermal cells are not so regular either in shape or size as those of the upper epidermis, nor are the outer walls quite so thick. The palisade parenchyma consists of a single layer of cells. These are narrow, much elongated, very regular in shape, and closely arranged. The cells of the spongy parenchyma are irregular in size and shape and so arranged as to leave rather large air spaces between the cells or groups of cells.

The lesions on the leaves are irregular in size and shape. The holonecrotic portion is black in color and is surrounded by a narrow, light green plesionecrotic border which blends into the normal green of the leaf.

When viewed in cross-section the black portion of the lesion is seen to consist of a stroma with a very dense black outer covering. On the upper surface of the leaf this stroma is only slightly elevated and beneath it the palisade cells are still in evidence, although they have shrunk and the contents have become disorganized. The hyphae are apparently intercellular. On the lower surface of the leaf the stroma is very much thicker and in some cases may cover a larger area than does the upper stroma. The contents of the spongy parenchyma cells which lie between the two stromata are usually wholly disorganized. Where the lower stroma covers a larger area than the upper, those parenchyma cells lying next to the stroma are disorganized while the remaining mesophyll cells may be perfectly normal. The hyphae do not appear to extend into the plesionecrotic zone and the contents of the cells in this region are only partially disorganized. There are apparently no histologic changes in the suscept which would prevent the further advance of the pathogene.

Lesions produced by Rhytisma ilices-canadensis Schw. on *Nemopanthus mucronata* (L.) Trel. Although similar in its general structure, the leaf of *Nemopanthus mucronata* is much thinner than that of *Ilex verticillata* and the upper epidermal cells are partially filled with a dense material resembling tannin.

The lesions are very similar in appearance to those just described on *Ilex verticillata* and in general the suscept reaction is the same. In the

sections examined, however, the hyphae extend a short distance into the plesionecrotic zone.

Lesions produced by Rhytisma acerinum (Pers.) Fr. on Acer rubrum L. The general structure of the leaf of *Acer rubrum* is the same as that of *Nemopanthus mucronata*. The appearance of the lesions and the type of suscept reaction are very similar to those described for *Ilex verticillata*.

Lesions produced by Mycosphaerella fragariae (Tul.) Lind. on Fragaria virginiana Duch. In the healthy leaf of this suscept the cells of both epidermal layers are large and rather broad. Many of the cells of the upper epidermis are partially filled with a material resembling tannin. The palisade parenchyma is composed of a single layer of compactly arranged cells which are rather short and taper slightly towards the lower end. The cells of the spongy parenchyma are irregular in size and shape and are very loosely arranged.

The lesions on the leaf are somewhat irregular in size and shape. In the older lesions the center of the holonecrotic area is grayish in color with a reddish-purple margin. This is surrounded by a wide plesionecrotic zone which is also reddish-purple in color, blending into the normal green of the leaf.

In the center of the holonecrotic area the cells have collapsed and the leaf is somewhat thinner than normal at this point. In the outer edge of this area the cells are filled with a brownish substance resembling tannin which failed to react to any of the stains used. These cells are not collapsed. In the plesionecrotic zone all of the epidermal cells are filled, or at least partly filled, with some material which stains deeply with haematoxylin. The contents of the mesophyll cells of this region stain more intensively than do the normal cells. The chloroplasts, although present, appear to be degenerating. The hyphae are present between the cells in the holonecrotic area but no trace of them can be found beyond the zone of mesophyll cells which are filled with the tannin-like substance. In no case is there any evidence of cicatrice formation although occasional cells at the outer edge of the holonecrotic area may be slightly enlarged.

When leaves of *Fragaria virginiana* are wounded a cicatrice is formed about the edge of the wounded portion. Multiplication and enlargement of the mesophyll cells occurs. All of these cells are thick-walled and the walls of the outer row are suberized. There is no evidence of a wound periderm such as occurs in *Prunus sp.*, but all of the cells are filled with the same granular substance found at the edge of the lesions produced by *Mycosphaerella fragariae*.

Lesions produced by Mycosphaerella grossulariae (Fr.) Lind. on Ribes prostratum L'Her. In a healthy leaf of *Ribes prostratum* the upper epi-

dermis is somewhat thicker than the lower. The walls of the upper epidermal cells are considerably thickened, and this thickening is present to a lesser extent in the walls of the lower epidermal cells. The palisade parenchyma is well-defined and is made up of a single layer of elongated cells closely packed together. The spongy parenchyma is composed of cells of various shapes and sizes which are loosely arranged.

The lesions on this susceptible are small and rather regular in outline. The holonecrotic center is grayish-white in color and is bordered by a narrow purplish-black plesionecrotic zone.

The holonecrotic portion is very little thinner than is the normal portion of the leaf. In this area many of the cells, both in the palisade and in the spongy parenchyma, have become filled with a dense granular substance resembling tannin. Such cells have only partially collapsed. All other cells in this area are apparently empty. The epidermal cells are collapsed and this condition is quite common even in the plesionecrotic zone. The contents of the mesophyll cells in this zone are becoming disorganized and the cells are filling up with the dense material already referred to. The intercellular hyphae extend for a short distance into the plesionecrotic zone. There is no evidence of the formation of a healing tissue about the edge of the lesion.

Lesions produced by Sphaerella tussilaginis Rehm. on Tussilago farfara L. In the healthy leaf of *Tussilago farfara* the upper epidermis is much thicker than the lower, the cells are larger, and the outer walls are much thicker. While the upper epidermal cells are arranged above the palisade cells in the ordinary way, the lower epidermis is decidedly wavy in its appearance. It appears as a chain of cells attached to the spongy parenchyma at regular intervals, each loop of the chain forming the outer boundary of a large air space. The palisade parenchyma is composed of about four layers of cells, the cells of the upper layer being much shorter and more regular in shape than those of the lower layers. These cells are closely packed, no intercellular spaces being visible in cross section of the leaf. The cells of the spongy parenchyma vary from almost square to very narrow and much elongated and are arranged in chains or groups. To the lower part of these chains, or groups of cells, the lower epidermis is attached.

The lesions on the leaf are circular in outline except when they are bounded by large veins. In the older lesions the holonecrotic center is grayish in color, and this is surrounded by a dark purple plesionecrotic zone of varying width. The edge of the lesion is not well-defined.

In the holonecrotic area the cells are entirely empty and the cell walls have collapsed. The leaf is about one half its normal thickness at this point.

In the plesionerotic zone the chloroplasts have broken down and disappeared but otherwise there seems to be little disorganization of the cell content. The upper layer of palisade cells and the cells of the upper epidermis contain some substance which stains deeply with haemotoxylin. No trace of the fungus was found outside of the holonecrotic area nor was there any evidence of cicatrice formation.

Lesions produced by Gnomonia leptostyla Fr. on Juglans cinerea L. The leaf of *Juglans cinerea* is very thin. The majority of the cells of the upper and all of those of the lower epidermis are narrow, long, and irregular in outline. In the upper epidermis, however, are found either single cells or groups of cells which are much larger than the others and either globose or oval in shape. The palisade parenchyma consists of a single layer of cells which are rather short and slightly narrowed at the lower end. The spongy parenchyma is made up of two or three layers of loosely arranged cells. The cells of the upper layer are large and resemble those of the palisade layer while the cells of the lower layers are very narrow and elongated.

The lesions on the leaf are somewhat variable in size and frequently unite to form large necrotic areas. The holonecrotic area is brownish-gray in color. The extreme margin of this area is really plesionecrotic in character.

In the holonecrotic area the leaf is very little thinner than normal. The epidermal cells and those of the spongy parenchyma are empty and the walls have collapsed. The palisade cells are partially filled with some dense granular material. These cells have apparently been reduced in length but the walls have not collapsed to the same extent as have the walls of the other mesophyll cells. In the plesionecrotic zone the contents of the cells are only partially disorganized. The hyphae are not abundant in the diseased region, but occasional threads are visible between the cells in the holonecrotic area and these extend to some extent into the plesionecrotic zone. This zone blends gradually into normal tissue.

Lesions produced by Guignardia bidwellii (Ell.) V. et R. on Ampelopsis tricuspidata Planch. In the healthy leaf of *Ampelopsis tricuspidata* the upper epidermis is composed of large cells which are, for the most part, somewhat oval in outline. The outer walls are very thick and the radial and inner walls, while somewhat thinner, are relatively thick as compared with the walls of the mesophyll cells. The cells of the lower epidermis are more irregular in size than those of the upper and the walls are markedly thickened. The palisade parenchyma is well-defined and is made up of two layers of cells. Those of the upper layer are long, narrow, and closely packed, while those of the lower layer are considerably shorter, somewhat

broader and more loosely arranged. The cells of the spongy parenchyma are variable both in size and shape. Some are almost rectangular in outline, others are almost globose, while still others are narrow and much elongated. These cells are rather loosely arranged and the intercellular spaces are fairly large.

The lesions on the leaves are variable in size and are usually irregular in shape. They frequently coalesce to form large necrotic areas and in extreme cases the greater part of the leaf may be affected. The holonecrotic center is brown in color with a sharply defined, lightly raised, purple-black margin which is plesionecrotic in character.

In the holonecrotic area the palisade cells have not collapsed completely but are much reduced in length. Many of these cells are filled with a dense granular material resembling tannin. The cells of the spongy parenchyma are for the most part empty and shrunk. In the plesionecrotic zone the cells have lost their chloroplasts although the cytoplasm is still in evidence (Fig. 9). In this region the cells of the upper layer of palisade parenchyma are slightly longer than normal and the upper epidermal cells are greatly enlarged. In the latter cells the contents have either disappeared or they are densely granular. The remainder of the mesophyll cells and those of the lower epidermis are apparently normal in size although their contents are somewhat broken down. As considerable difficulty was experienced in staining the mycelium, no satisfactory evidence was secured relative to the abundance of the hyphae in the holonecrotic area nor the distance to which it had penetrated the plesionecrotic zone.

Artificial wounding of the leaves of this suspect resulted in the formation of a definite cicatrice (Fig. 10). This cicatrice is of the same general type as that produced on the leaves of *Prunus domestica* when similarly treated.

Discussion

While the leaf tissues of the suspects included in this group do not respond to the invasion of the pathogene by the formation of a definite cicatrice, nevertheless the cells about the holonecrotic area are far from being normal in character. The changes occurring in these cells are mainly disorganization of the protoplasm and the collapse of the cells. The extent or degree of such changes varies in different plants and seems to be associated with the character of the suspect rather than the pathogene invading the tissues.

In some leaves the cells in the holonecrotic area may be entirely empty and collapsed, a condition found in *Apium graveolens* when attacked by *Septoria petroselinii*, and as a result this portion of the leaf may be much

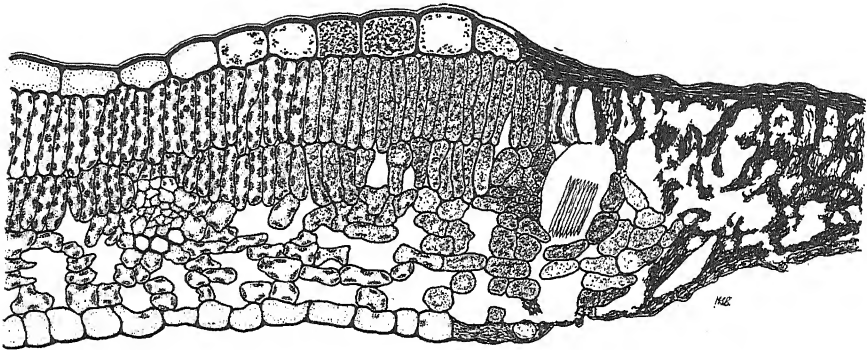


FIG. 9. Cross section through a diseased leaf of *Ampelopsis tricuspidata* showing the histologic changes induced by *Guignardia bidwellii*. $\times 230$.

thinner than normal. On the other hand many of the cells in this area may be partially or wholly filled with a dense granular material as is the case with leaves of *Steironema ciliatum* attacked by *Septoria conspicua*. In such leaves many of the palisade cells and some of the cells of the spongy

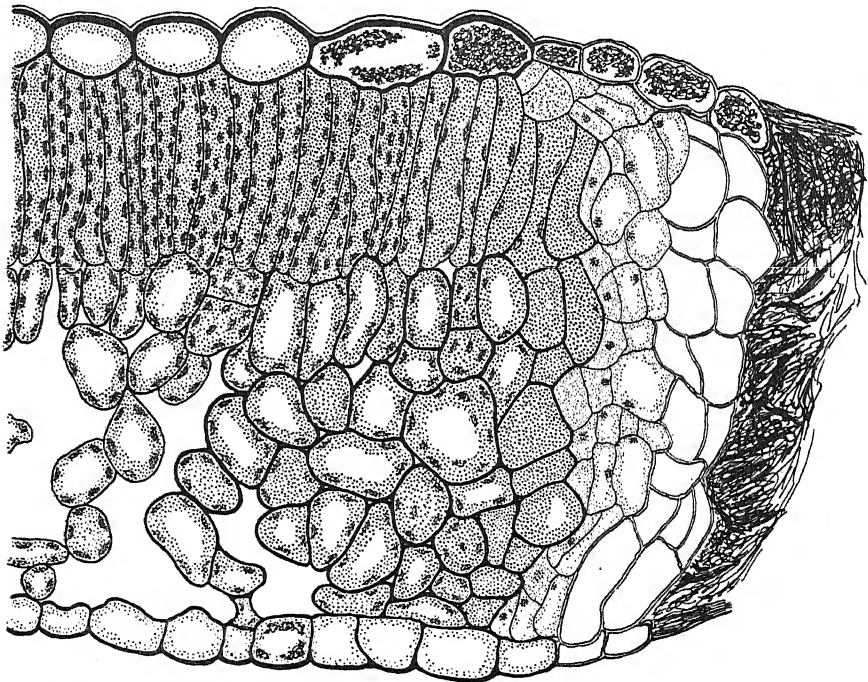


FIG. 10. Cross section through the edge of a wound on the leaf of *Ampelopsis tricuspidata* showing the cicatrice formed under these conditions. $\times 321$.

parenchyma contain this substance and as a result little or no shrinking of the necrotic tissues has occurred.

The extent to which the hyphae of the pathogene penetrate beyond the holonecrotic area differs greatly in the leaves of the plants studied. Not only does this vary with the different plants but it varies in different lesions on the same leaf and is affected in many cases by the presence or absence of veins. The extent of this hyphal invasion is important in its effect upon the cells in the invaded region for such cells invariably show the influence of the pathogene by a disorganization of the protoplasm, followed later by a partial or total collapse of the cells. In leaves of *Apium graveolens* attacked by *Septoria petroselini* the hyphae extend for a long distance beyond the holonecrotic area, as reported by Rogers (16), and the cell contents in this region are more or less disorganized. The same condition is found in leaves of *Primula polyantha* attacked by *Ramularia primulae*, and also in leaves of *Caulophyllum thalictroides* attacked by *Cercospora caulophylli*. On the other hand in leaves of *Symplocarpus foetidus* attacked by *Cercospora symplocarpi* the hyphae extend little if any beyond the holonecrotic area and only a few cells can be classed as plesionecrotic. A similar condition exists in leaves of *Ilex verticillata* attacked by *Rhytisma ilices-canadensis*, in leaves of *Medicago sativa* infected by *Pseudopeziza medicaginis*, and in leaves of *Solanum tuberosum* bearing lesions caused by *Alternaria solani*.

In the case of the last mentioned suspect it was thought that possibly the target-board effect caused by *Alternaria solani* might be due to the formation of a periderm as was shown by Hesler (6) to be the case in *Pyrus malus* attacked by *Sphaeropsis malorum*. Jones (10) and Rands (15) have suggested that this effect is caused by the epidermal cells being thrown into folds due to shrinking caused by the collapse of the mesophyll cells. Whatever may be the effect of this shrinkage of the mesophyll cells the writer's observations show that, for some unknown reason, the palisade cells of the target board lines are only partially collapsed while this collapse is complete in the cells between these lines.

CONCLUSIONS

The results reported in this paper show clearly that whatever may be the factor or factors governing the limitation of the necrosis caused by fungi in the leaf spot diseases of plants, it is by no means due in all cases to the formation of a cicatrice or healing tissue within the suspect, which limits the advance of the pathogene. In some plants the usual reaction of the leaf to pathogene injury appears to be the formation of a cicatrice. In a large majority of cases, however, there is no attempt on the part of the

suscept to lay down such a barrier. Neither does it follow that a plant which forms a cicatrice about mechanical wounds in its leaves will respond in the same way to localized necrosis caused by fungi. On the other hand all of the susceptibles studied during these investigations which did produce a cicatrice about a fungous necrosis also formed a cicatrice about mechanical injuries. Whether these plants react in the same way to the attacks of other leaf-spotting organisms remains a problem for future work.

SUMMARY

Leaves of *Prunus domestica*, *P. avium*, *P. cerasus*, and *P. virginiana*, when attacked by species of *Coccomyces*, form a definite cicatrice about the edge of the lesion, thus isolating the diseased portion from the healthy. The same reaction occurs when leaves of *Pyrus communis* are attacked by *Mycosphaerella sentina* and when leaves of *Beta vulgaris* are attacked by *Cercospora beticola*. Artificial wounding of leaves of these plants results in the formation of a cicatrice similar to that formed in diseased leaves.

No cicatrice is formed in either diseased or wounded leaves of *Podophyllum peltatum* or *Symplocarpus foetidus*. In wounded leaves of these plants the cells for some distance from the edge of the wound are dead and collapsed while the walls of a few cells adjoining the inner edge of this collapsed area are suberized.

Diseased leaves of *Fragaria virginiana* showed no evidence of cicatrice formation, but wounded leaves of this plant formed a definite cicatrice about the edge of the wound. These cells have thickened walls and are suberized in part while all of the cells are filled with a dense granular material. There is no evidence of a wound periderm such as is formed in wounded leaves of *Prunus domestica*.

Although diseased leaves of *Ampelopsis tricuspidata* do not react by the formation of a cicatrice, yet wounded leaves of the same plant form a well developed wound periderm about the edge of the injury.

Diseased leaves of all other susceptibles included in this investigation failed to form a cicatrice separating the lesion from healthy tissues. The type of reaction in these cases consists of a degeneration of the protoplasm, with or without granular deposits, together with more or less collapsing of the cells. No evidence of suberization of the walls of the cells at or near the edge of the lesions was observed. These leaves were not wounded and their reaction to such injuries is unknown.

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THREE HELMINTHOSPORIUM DISEASES OF SUGAR CANE

JAMES A. FARIS

In 1892 van Breda de Haan (1), a Dutch investigator working in Java, described a leaf disease of sugar cane, which, in its mature stage, causes a red to red-brown spot surrounded by a yellowish zone, the center of the spot drying up as the infection advances. Owing to the elongated elliptical shape of this spot, it was named "eye spot" by Krüger (1).

This disease is further described as elongating length-wise of the leaf in rather wide, yellowish bands from the typical spots, the spots and bands thereupon suggesting a peacock-feather in form. In the end, all the attacked leaf parts dry up and die, but it is still possible to find the original form of the spot again by holding the leaf toward the light, as the tail-like spot will appear lighter than the other parts.

Details are given as to the pathological anatomy of the diseased leaves. It is also pointed out that cane varieties differ greatly in their susceptibility to the disease, the Bali variety being so susceptible that large portions of the leaves are attacked at an early stage and the plants develop very poorly. On other varieties in which disease is limited to a few broad bands, the disease in most cases is not serious.

The influence of weather conditions is stated to be very great, the worst attacks coming at the end of the rainy season, and, under conditions ideal for the attacking fungus, the growth of the plants is slowed up to a considerable degree.

The cause of the disease was shown by infection experiments to be a fungus, which was hyaline outside the leaf tissue but developed a brown tint inside the leaf. The fungus was observed to produce dark brown conidiophores through the stomata; very seldom were they seen breaking through the epidermis to reach the surface. These dark brown conidia bearers were poly-celled and when fully grown measured 120–160 μ . From these, conidia are produced with 6–9 cells measuring 60–80 μ in length by 9–12 μ in breadth. Mature spores are described as having a lengthened oval form and a smoke-brown color. No illustrations are given of this fungus, but the organism was thought by van Breda de Haan to be a new species in the genus *Cercospora* and he named it *Cercospora sacchari*.

In 1894 Lucassen and Went (20), other investigators in Java, published a colored plate of the eye spot disease of sugar cane leaves, which they describe briefly, citing the article by van Breda de Haan for further

reference, and giving the fungus *Cercospora sacchari* van Breda de Haan as the causal organism. Their colored plate is very typical of the eye spot of sugar cane as I have observed it in Porto Rico, Santo Domingo, and Cuba; and specimens of eye spot in Cuba were identified by Hawaiian workers as identical with that of Hawaii. From this it seems clear that the disease described by van Breda de Haan is identical with the eye spot disease of cane which we are studying.

The colored plate of Lucassen and Went was again published in the well-known textbooks by Wakker and Went (23) in 1898 and by Krüger (15) in 1899. Both of these books contain drawings of spores of the eye spot fungus which show it to be a *Helminthosporium* rather than a species of *Cercospora*.

Butler and Hafiz (4) in 1913 reported upon some new sugar cane diseases in India and described a Helminthosporiose of sugar cane leaves at Pusa. Infected leaves are described as first showing "small red spots, which enlarge rapidly, chiefly in a longitudinal direction, and, especially toward the tip of the leaf, may run together to form long streaks. The center of the spot soon changes to a dirty straw color, around which the margin remains red for a time and then becomes dark brown." Reference is then made to a colored plate showing two elongated spots with very irregular borders, with no yellowish halo but with a very narrow, line-like, red border separating a broad, straw-colored center from the green tissues of the leaf. If this spot is typical of the disease these authors had under observation, it could not be mistaken for the eye spot figures by Lucassen and Went (20) and Krüger (15) in Java, by Bruner (2) in Cuba, Cobb (5) and Lee (16) in Hawaii, Cook (8) in Porto Rico, or for the eye spot disease we have observed on sugar cane in Porto Rico and Santo Domingo, and which is being studied at present in Cuba. Butler and Hafiz (4) state also that the spots of their Helminthosporiose occur equally on the thin part of the leaf and on the midrib, but in this the disease differs from eye spot, which seldom occurs on the midrib except in heavy infection on very young plants. Lee and Martin (18) state, "the midrib is rarely attacked (by the eye-spot fungus), if at all, in the case of No. 109." Butler and Hafiz (4) then described the fungus as having sporophores 100–190 μ in length by 5.5–7.5 μ in diameter bearing spores 3–10 septate and 35–60 μ in length by 8.5–12 μ in diameter, and named it *Helminthosporium sacchari*. The following statement is then added: "A fungus which, from published descriptions, bears a considerable resemblance to the above, is known in Java and Hawaii under the name of *Cercospora sacchari* Br. de Haan. It was first described by Breda de Haan in Java, as the cause of a leaf disease to which the name

'eyespot' was given. . . . From figures published in Wakker and Went's well-known textbook of sugar cane diseases, it appears probable that this fungus is a *Helminthosporium* and not a *Cercospora*. A comparison of the two fungi has not been possible and could alone settle the question of their identity."

In 1917, Johnson and Stevenson (13) made *Helminthosporium sacchari* Butler a synonym of *Cercospora sacchari* van Breda de Haan. The fungus was then described as having conidiophores 120–160 μ long bearing spores 32–90 $\mu \times$ 9–14 μ . No mention was made of a comparative study of the fungi, such as Butler and Hafiz (4) believed "could alone settle the question of their identity." In a later publication these authors, in collaboration with Ashby, Bancroft, and Nowell (14) do not seem to have been certain of the identity of *H. sacchari* Butler and *Cercospora sacchari* Br. de Haan, as they add, after quoting Butler's description of the former, "Probably identical with *Cercospora sacchari*, Br. de Haan, which from illustrations does not appear to be a *Cercospora* but an *Helminthosporium*."

The organism causing eye spot in Porto Rico, Santo Domingo, and Cuba agrees fairly well as to conidiophore measurements and spore measurements with those given for *Cercospora sacchari* Br. de Haan.

Likewise measurements by other authors who have given figures for the eye spot organism seem to agree fairly closely with those given by Breda de Haan, as may be seen from table 1.

TABLE 1.—Spore measurements of *Helminthosporium* on sugar cane

Author	Length	Average	Width	Average	Gen. average of spore measurements
<i>Eye Spot Helminthosporium</i>					
Breda de Haan, Java (1).....	60–80 μ	70 μ	9–12 μ	10.5 μ	70 \times 10.5 μ
Johnston & Stevenson, Porto Rico (13)	32–90 μ	61 μ	9–14 μ	11.5 μ	61 \times 11.5 μ
Cook, Porto Rico (6).....	45–110 μ	77.5 μ	12 μ	12 μ	77.5 \times 12 μ
do (8).....	22–92 μ	58.7 μ	6.6–15 μ	11.1 μ	58.7 \times 11.1 μ
Drechsler (9)	32–103 μ	71 μ	9–17 μ	14 μ	71 \times 14 μ
Faris, Cuba	29–84 μ	68.9 μ	9–21 μ	12.7 μ	69 \times 12.7 μ
<i>Helminthosporium sacchari</i>					
Butl. (3, 4)	35–60 μ	47.5 μ	9.4–12 μ	10.7 μ	47.5 \times 10.7 μ

According to these measurements all authors agree that the spores of the eye spot organism are, on the average, much longer than those given for *Helminthosporium sacchari* by Butler and Hafiz (4) and Butler (3). In fact, five of the six measurements given show the average length of the

spores of the *Helminthosporium* causing eye spot to be greater than the maximum length of the spores of *Helminthosporium sacchari* Butler.

In order to make a comparison of the Helminthosporiose of sugar cane in India with the eye spot in Cuba, a fragment of the type material of the former was solicited from Dr. E. J. Butler. None was available, but Dr. Butler referred us to the Hawaiian Sugar Planters' Experiment Station where authentic material from India could be secured. Through the courtesy of the officials of that station, a specimen was obtained of Helminthosporiose on *Saccharum officinarum* from Pusa, India, collected in December, 1924, by Azambullah Kahn. The material was preserved in 4 per cent formalin solution; hence no cultural experiments could be made.

The spots on the material received are of the type described and figured by Butler and Hafiz (4), and resemble, in the early stages, the well-known ring spot more than they do the eye spot. They differ from the ring spot, however, in having a wider ring of red tissue, which Butler and Hafiz (4), and Butler (3) describe as later becoming dark brown. Since the material received is preserved in formalin, too much dependence should not be placed on the color values. The center of the spots are grey and are covered with conidiophores and conidia of *Helminthosporium*. Spores from these spots varied from $9.40\ \mu$ to $15.00\ \mu$ in width (weighted average $12.54\ \mu$) and in length from $18.80\ \mu$ to $71.44\ \mu$ (weighted average $52.60\ \mu$) with an average of 5.5 septa.

Taking into consideration the material in hand, the description of the Helminthosporiose of sugar cane by Butler and Hafiz (4), and the characters given by Butler for *Helminthosporium sacchari*, I am brought to the conclusion that the Helminthosporiose and eye spot are two distinct diseases and are caused by different species of *Helminthosporium*.

This conclusion is based upon the following observations: a) the disease pictured with a colored plate by Butler and Hafiz (4) and by Butler (3), a specimen of which I have in hand in no way resembles eye spot in appearance; b) the organism causing eye spot has spores on the average $20\ \mu$ longer than those of *H. sacchari* Butler.

Therefore it is a fundamental error to attribute eye spot to *Helminthosporium sacchari* Butler, which causes an entirely different disease and has different mycological characters. As pointed out by Butler and Hafiz (4) and Butler (3), the organism causing eye spot is, without doubt, a *Helminthosporium*. From the present studies it is clear that it is not *Helminthosporium sacchari* Butler.

As the situation now stands the well-known and destructive eye spot disease is due to a species of *Helminthosporium* having fuliginous thin-walled conidia 3-10 septate, measuring $32-110\ \mu$ in length (average $69\ \mu$)

by 6.6–20.8 μ in width (average 12.7 μ), which are not identical with those of *H. sacchari* Butler. Nor are they identical with conidia of *H. stenospilum* Dreschler (9), which causes the brown stripe recently described by the writer (10) as a new *Helminthosporium* disease of sugar cane.

The organism causing eye spot of sugar cane is provisionally named *Helminthosporium ocellum* n. sp.

Helminthosporium ocellum n. sp.

Occurring on the leaves of *Saccharum officinarum*, on many varieties of which it causes spots of a Bordeaux red (Ridg.) with the color of the center very much diluted at the edges, usually surrounded in the younger stages by a marked halo. Conidiophores of a smoky yellow-brown color, 3–8 septate (the septa generally occurring at intervals of 15–45 μ); 3.5–5 μ in width by 145–380 μ in length; points of attachment of successive spores marked by moderately pronounced geniculations.

Conidia subhyaline, light smoky yellow-brown; mature spores typically slightly curved, with the bulge in the middle third of the spore; maximum diameter, usually near the middle, 9–21 μ (weighted average 12.7 μ); length 29–94 μ (weighted average 69 μ); typically 3–10 septate (average 6.7).

Germination is typically by two germ-tubes, one from each end of the conidium.

Habitat—On *Saccharum officinarum*, collected by the author in Cuba, Porto Rico, Santo Domingo. This fungus, isolated from Florida material, was received in pure culture from Dr. Chas. Drechsler, Nov., 1927, and has been collected in Florida by B. A. Bourne (1927). It has also been reported from Hawaii and Java by various authors.

This species of *Helminthosporium* differs from *H. sacchari* Butl. in having longer, lighter-colored conidiophores and conidia, and from *H. stenospilum* Drechsler in having shorter conidia of a much lighter color (Fig. 5). The leaf spots produced by the three species also have very distinctive characters, as may be seen from the description and illustrations.

The *Helminthosporium* diseases of sugar cane present some important problems for investigation both in regard to their economic importance and from the standpoint of the fungi causing them. A *Helminthosporium* spot, which has previously been considered an immature stage of the well-known eye spot, has proved to be a distinct disease and was briefly described (10) as brown stripe of sugar cane. Table 2 gives the outstanding characteristics of the three diseases.

The discovery of this error in considering the *Helminthosporiose*, brown stripe, and eye spot diseases as a single malady raises the interesting ques-

TABLE 2.—*Diagnostic characters of eye spot, helminthosporiose, and brown stripe diseases of sugar cane*

Character of spotting	Eye spot	Helminthosporiose	Brown stripe
	Bordeaux red color, usually with a marked halo in the young stages. In very susceptible varieties elongating into long tail-like streaks from a single spot to the tips of the leaves. The centers of old spots darken to a dark purplish Bordeaux red and the edges to a burnt sienna.	Red border with straw colored center extending into long streaks by the coalescing of various spots. The margin of the spot is red and changes to a dark brown. Very little, if any, halo outside the red border.	Vandyke brown color with a scant yellowish green halo. In very susceptible varieties the spots elongate by increase of the spot itself rather than as tail-like elongations. The color darkens in older spots, the centers of which have a grayish cast.
Conidiophores	Profusely produced on older leaves throughout diseased area, 145–380 μ long, prostrate, simulating a surface mycelium over the peacock-feather-shaped spots. Marked bulbous base, smoky yellow-brown color.	Profusely produced in the grayish center of the spots, 100–190 μ in length, short, stout, erect, rather rigid. Color dark greenish-brown, paler above.	Sparsely produced over the brown area of the stripes. Color dark-olivaceous. Length medium, 120–260 μ , erect, rather rigid, slightly bulbous at base.
Spores	Light smoky yellow-brown, 12.7 μ \times 69 μ , typically slightly curved.	Olive-green to brown, 8.5–12 μ \times 35–60 μ , typically cylindrical or long elliptical in shape.	Dark olivaceous color, 17 μ \times 84 μ , typically uniform.

tion as to the correctness of reports upon the world distribution of these various cane diseases. See Plate XV.

EYE SPOT IN CUBA

Herbarium material at the Estación de Santiago de las Vegas shows that eye spot was collected on sugar cane in that vicinity April 5, 1915, by J. R. Johnston; February 15, 1918, by S. C. Bruner; and June 12, 1920, by S. C. Bruner.

Bruner (2) has also reported this disease, which he states is of a linear form in the cane in Cuba, measuring from 5 to 12 by 1 to 2 mm.

Both the eye spot and brown stripe diseases are present at Santiago de las Vegas and, since the brown stripe was considered an immature stage of the eye spot until my investigations were reported upon (10) in March, 1927, it is quite likely that this reference to the linear form of the spot refers to brown stripe rather than eye spot.

Owing to the great resistance of Cristalina cane to infection by the eye spot fungus, this disease has been of minor importance in Cuba. However, the introduction from other countries of new varieties which are susceptible to the disease has brought it to the attention of planters. The disease also has been reintroduced with large importations of cane, and we now find it widely distributed over the island. It is present and widely distributed in Oriente Province, present in at least one place in Camagüey and Matanzas Provinces, and in several places in Havana Province. It is present in at least one locality in Santa Clara Province and probably occurs in Pinar del Rio also.

Varietal Susceptibility to Eye Spot

Lee, Martin, Stender, and Barnum in various papers (16-19 and 21) report H 109 cane to be very susceptible to eye spot in Hawaii. Cook (6) in a recent paper reports upon the susceptibility of a number of canes to this disease in experimental studies and field tests, and his results in Porto Rico agree very closely with mine secured during the outbreak of this disease in Cuba in the winters of 1926-1927 and 1927-1928.

The varieties attacked most vigorously were the following Fajardo Central seedlings: F. C. 136, F. C. 214, F. C. 306, and D 109. The last two varieties were being extended to field scale plantings in Oriente Province, Cuba, but, owing to their great susceptibility to this disease, they are not now being extended as field canes. Table 3 compiles results of natural infections in varietal plots in eight different localities. In no case was the widely grown Cristalina cane suffering losses from this disease, although a severe epidemic had developed in recently imported varieties growing in adjacent fields. In all cases the outbreak of the disease was very severe, and it is believed the grouping is a true representation of the reaction which may be expected from the varieties listed under Cuban cultural conditions.

A more extensive list of varieties would be of interest, but these data include all those planted on the eight plantations visited.

Environmental Factors Influencing Infection

The characteristic spots of this disease can be found in affected areas throughout the year but the disease develops very slowly during the periods of hot and dry weather. The cool rainy weather during the winter months usually brings on a severe epidemic in susceptible varieties. During the drier winter months the epidemic gathers force in pockets and valleys where the dew and fog keep the leaves damp until late in the morning. The disease develops vigorously on rapidly growing cane, differing somewhat in this respect from brown stripe which develops better upon cane of slow

TABLE 3.—*Relative susceptibility of cane varieties to eye spot disease in Cuba*

GROUP I.—Varieties which are very susceptible, that is, in which the spots develop very quickly into reddish streaks to the tip or edge of the leaf. The growth is checked, the leaves quickly dry, and sometimes the disease extends throughout the entire top of the plant, resulting in a top rot.

D 109	F C 214
F C 137	F C 306

GROUP II.—Varieties in which spots develop into reddish streaks but which are not so intense in color as in Group I.

Guanica seedling	D 216
H 109	POJ 36
D 433	P R 417
	POJ 100

GROUP III.—Varieties in which spots are produced abundantly but in which, although conditions were the same as for Groups I and II, streaking was absent or very rare.

B 3412	P R 440
B 6308	P R 492
C 35	Sealey seedling

GROUP IV.—Varieties in which mostly spots are produced but not so plentifully as in Group III.

C 553	F. C. 299
General Betancourt	P R 328
Yellow Caledonia	B 3405
Caña Blanca	P R 724
D 1135	P R 725

GROUP V.—Varieties in which spotting is rare, even when growing near heavily infected varieties.

B H 10 (12)	POJ 2714	C 219
B 4596	POJ 2725	P R 700
B 6032	P R 358	P R 16/13
EK 28	S C 12 (4)	P R 707
P R 209	Cristalina	
	Tjepering	

GROUP VI.—Varieties which are resistant and in which very little, if any, spotting was observed. When spots appeared they were small flecks.

D 117	POJ 2727	POJ 36
Java 105	Uba	Kavangire
Java Unknown	Co 281	Cuba 64/21
Badila	D 116	B 1809
POJ 234		B 1753

growth at any time of the year, the outbreaks being much more severe during the dry period of July and August.

Control Measures

It is probably unwise to plant any of the varieties of Group I in Cuba, as a combination of environmental conditions may occur which would cause very serious loss. If canes of Group II are planted, there may be considerable loss in the more humid regions because of the eye spot disease. The fungus is so widely distributed that the susceptibility of any new varieties must be determined before their planting on a field scale can be safely undertaken.

BROWN STRIPE OF SUGAR CANE

During the fall of 1924, a brown linear striping was observed throughout Cuba upon Cristalina cane. From these stripes it was seldom possible to secure sporulating material from the field before the leaves had dried. From spots on dried leaves a *Helminthosporium* could easily be secured. Small blocks of the brown striped areas were taken from green leaves, surface sterilized, and then plated in cornmeal agar. In a very large percentage of cases pure cultures of a *Helminthosporium* were secured, the spores of which were considerably larger than those described by Butler for *H. sacchari*. However, since it was generally stated that the *Helminthosporium* on sugar cane was *H. sacchari* and since the leaf disease under observation resembled to some extent immature stages of the eye spot disease, no serious attention was given to it until late in the season of 1925 and during 1926. During the dry seasons of these two years the outbreak on Cristalina cane, the chief commercial variety of Cuba, was so severe that in some sections of the island both the young and old leaves were thoroughly covered with the characteristic elongated narrow spots or stripes. The variety B H 10 (12) was also badly attacked in the fields of the Experiment Station at Central Baraguá, and S. C. 12 (4) also proved to be rather susceptible, the spots being somewhat wider and longer than is the case on Cristalina and B H 10 (12).

Even though some experiments were under way at that time, it was still considered that this disease was probably immature eye spot. However, a serious outbreak of eye spot in Oriente Province, Cuba, in the winter of 1926-1927 indicated that these were two distinct *Helminthosporium* diseases on sugar cane. There was a marked difference in varietal susceptibility to the eye spot, the varieties D 109, FC 214, and FC 137 being so seriously attacked that the leaves were drying and in many cases the tops of the

plants were rotting, while in Cristalina fields alongside it was very difficult to find even the least trace of eye spot.

Four variety collections in this province, all of which came originally from Porto Rico, were then visited, and a heavy outbreak of eye spot was found in each of them. Cristalina cane growing alongside these heavily infected areas showed only the slightest traces of eye spot, and in one case none at all.

On the contrary, in two of our experimental plantings, one in Oriente Province and the other in Camagüey Province, we found the brown striping very plentiful on Cristalina as well as on many other varieties, but no eye spot was found. Close observation showed marked differences in the eye spot and brown stripe diseases, not only as to the susceptible varieties but also in the shape, form, and color of the spotting, and in the characters of the causative fungi, in average spore measurements and type of growth upon media.

Cook (7), in July, 1924, published a short note on leaf spot of sugar cane in which he thought he had two spot diseases due to *Helminthosporium*. One of these he termed the "Manati disease." The other he finds occurs only on B H 10 (12) cane at Guanica in Porto Rico. In October of the same year (6) he published a preliminary paper describing the *H. sacchari* in Porto Rico as being subject to "considerable variations which may be due to local conditions or to varieties of host plants or to unknown causes; that the Manati form is the same or a closely related species and the Santa Rita form is a variety or possibly a new species. However, it may be that the Santa Rita disease¹ as described may be due in part to other causes." As described, the spots of the Santa Rita disease fuse to form blotches of a red to dark purple color, the purple predominating and increasing to cover more than half the lower part of the leaf, while the "upper or outer half of the leaf shows very little or no spotting but with the advancement of the disease on the lower half it becomes yellow and ashy brown. The sheath is finally attacked but not until the disease is well advanced on the blade."

From this description we were certain that our brown stripe was not the same as this Santa Rita disease. In a recent paper, Cook (8) states that the two names given above have gone out of use and that the disease is now generally known as eye spot. He then states: "The first studies made by the writer showed considerable variations in the spots and in size of the spores which led us to suspect that the spotting might be due to two species of organism causing the disease but later studies have shown that this is not true."

¹ The disease on B H 10 (12), according to Faris.

GROWTH CHARACTERS OF THE FUNGI CAUSING BROWN STRIPE AND EYE SPOT

Parallel isolations and platings were made of both species of *Helminthosporium*, that causing the well known eye spot and that of the brown stripe, in order to make comparative studies.

Blocks of leaf tissue showing the typical brown stripes and eye spots were surface sterilized in 95 per cent alcohol, flamed and then plated in cornmeal agar (Shear's formula), cane juice agar (2 per cent cane juice), and 1 per cent sucrose agar. A very large percentage of such platings yielded pure cultures of the organism. From sporulating material of such cultures platings were made and single spore cultures were secured. Within a period of 24-48 hours these fungi sporulate freely in cornmeal agar. However, the growth characters of the colonies are more constant and distinctive on 1 per cent sucrose agar.

The growth of these two fungi is well illustrated in figures 1 and 2, which are photographs of cultures 18 days old on 1 per cent sucrose agar plates. Single spore cultures of these two fungi isolated and reisolated to

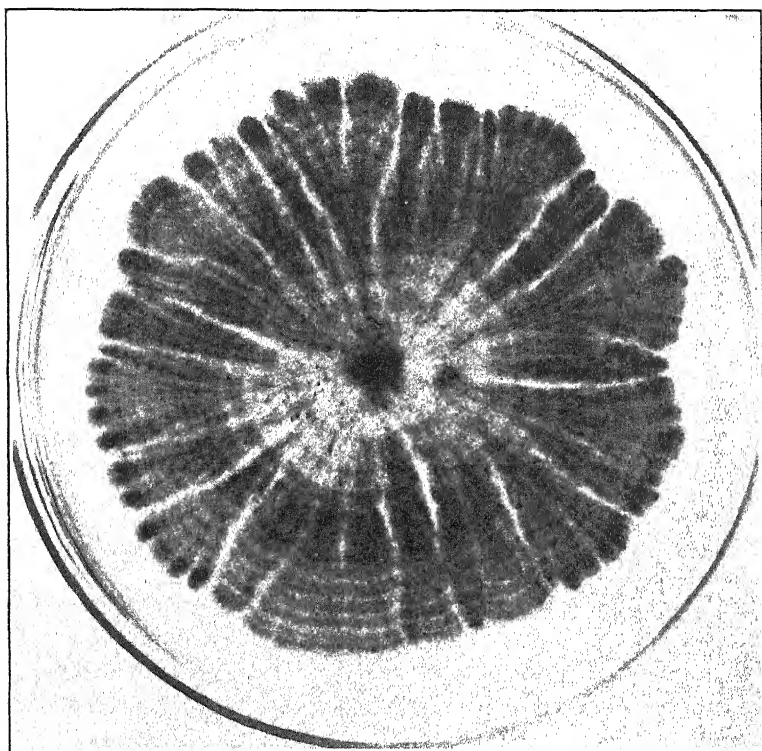


FIG. 1. Culture of eye spot *Helminthosporium* 18 days old on 1 per cent saccharose agar.

the fifth generation maintain these characteristic differences when grown on this medium and incubated at 25° C. On cornmeal agar and cane juice agar the growth characters were not so distinct.

The *Helminthosporium* causing brown stripe forms marked concentric rings, the hyphae growing radially from the spore or block of infected tissue, but lateral branches mingle and cross freely. The concentric rings formed are much broader than those of the eye spot fungus grown under the same conditions, and more nearly circular in outline. The concentric rings of the latter are rather irregular, owing to the type of growth which simulates dichotomous branching. Here again the growth is radial from the spore or block of infected tissue, but the hyphae soon divide and form sectors which seem to be repellent to one another and the lateral hyphae do not cross freely, thus leaving radial strips of agar between the sectors of fungous growth. This division continues into subdivisions, and a simulation of dichotomous branching is secured. These differences are clearly shown in figures 1 and 2.

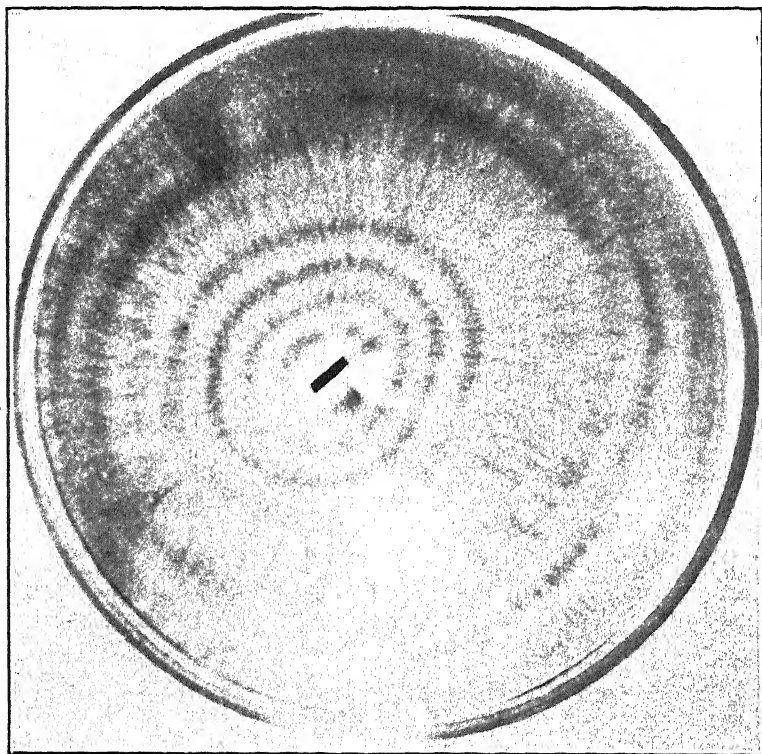


FIG. 2. Culture of brown stripe *Helminthosporium* 18 days old on 1 per cent saccharose agar.

While there are considerable differences in the growth of these two fungi on various media, when they are grown upon 1 per cent sucrose agar (2 per cent agar-agar) from single spore cultures, the broad concentric rings and free intermingling of lateral hyphae of the brown stripe organism are always in great contrast to the narrow concentric rings of broken outline, and sectorial growth of the eye spot *Helminthosporium*.

INFECTION EXPERIMENTS

The great profusion of brown stripe in the fields of Cristalina cane close to the laboratory made it impossible to use plants grown in the open for inoculation experiments. All plants used for studies in comparing the types of spots produced by the two *Helminthosporiums* in the early stages were germinated in chambers protected from outside spores. This was done in two ways. The simpler and less accurate method was to keep the pots in a chamber covered with a double layer of cheese-cloth and to make inoculations under a bell jar in this chamber.

This method was supplemented by the more accurate one of growing the plants under sterile conditions and making inoculations from pure single-spore cultures under aseptic conditions. Since this method presents some new features for the study of cane diseases, it is described somewhat in detail.

In glass cylinders 50 cms. high by 10 cms. in diameter was placed about 500 gms. of sand, moistened to about 70 per cent of its moisture-holding capacity with Schimper's normal culture solution. The cylinders were then stoppered with cotton, wrapped in paper, and sterilized 5 hours at 25 lbs. steam pressure in a large autoclave. At the same time smaller tubes holding 200 gms. of moistened sand were sterilized and the sand in these used to cover the cane when planted in the large cylinders.

Good seed pieces of the variety desired were thoroughly cleaned of all leaf parts, dirt, etc., and then immersed in water at 53.5° C. for 20 minutes, dipped in 95 per cent alcohol, soaked in 1-1000 HgCl₂ for 10 minutes and then planted without rinsing.

The cylinders were placed beneath a sterile hood and the paper wrapping removed. The seed cane was transferred from the mercuric bichloride solution into the sterilized glass cylinders under an aseptic hood, and a smaller tube of sterilized sand was used to cover the seed piece in the glass cylinders. In about 3 weeks the cane in these cylinders was ready for inoculation. A very high percentage of plants grown in this way were free from any contamination. In figure 3 the details of the apparatus described above are shown.

The jars were placed in the plant house for a period of 3 or 4 weeks until the plants had produced 5 or 6 leaves. They were then again transferred

to the aseptic hood and inoculated by means of a sterilized atomizer with single spore cultures of the *Helminthosporium* being studied. Great care was used to avoid contamination of any kind, and cultures which became contaminated were discarded.

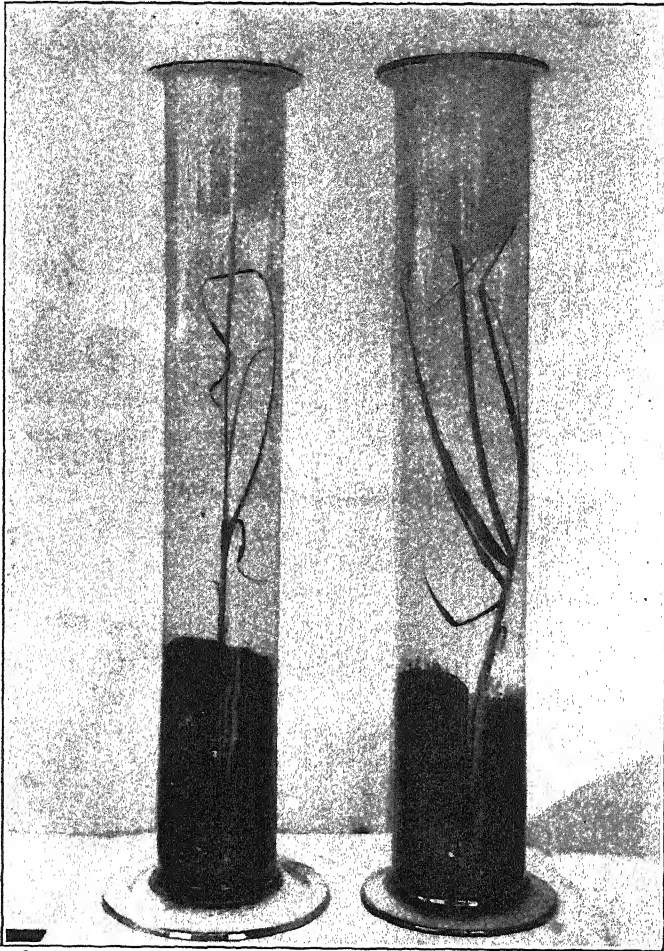


FIG. 3. Sugar cane plants growing under aseptic conditions.

By this method it was comparatively easy to demonstrate quickly and definitely the differences in the types of spots produced by the two species of *Helminthosporium*. The cultures under aseptic conditions were supplemented by pot cultures grown under cheese-cloth and inoculated by spray-

ing pure cultures of the organism and then placing bell jars over the plants for a period of three days.

It is believed that spores from diseased tissues produced under these aseptic conditions in a saturated atmosphere will be more comparable than when taken either from cultures, field material, or field collections placed in a moist chamber. That is, infected tissue killed by the parasite while attached to the plants, under conditions of saturated humidity and not in competition with other parasites, affords more nearly constant and comparable conditions than is secured by the methods usually employed in identifying and describing parasitic fungi.

Parallel experiments with single spore cultures of the fungi from eye spot and brown stripe were run.

Both types of spotting began to appear as very minute yellowish flecks within 48 hours. After 72 hours the spots were very numerous and had developed a reddish center. From this time on it was possible to distinguish between the two diseases on the F. C. 306 variety. The brown stripe became darker and elongated slowly to a definite linear stripe surrounded by a definite, yellowish green halo of fairly uniform width. This halo was, in general, about the width of the stripe and followed more or less the outline of the darker central portion, with perhaps a little greater width at the ends of the elongated spots.

The eye spot, on the other hand, did not turn so dark brown but remained of a brick-reddish cast. The spots were wider in relation to their length, but the most distinctive difference was the indefinite margins of the reddish central portion of the eye spot as compared with the definite margins of the dark brown central portion of the brown stripe, and the wider and more irregular halo of the eye spot. This wider halo is markedly longer in the lengthwise direction of the leaf, extending both ways from the longer axis of the spot, but soon develops long, lighter colored rays toward the tip of the leaf, which later become reddish to form the typical peacock-feather-like stripes as were described by van Breda de Haan (1). The brown stripe has never shown any tendency to extend in these long rays from the spots, even in the most susceptible varieties, such as *Cristalina* and B. H. 10 (12).

The differences described above are the same as those shown in the field by the two diseases. The plants grown in the jars are smaller and the spots are correspondingly smaller. The following description of the spots is made from field material of F. C. 306 and D 109, both of which are susceptible to both diseases (Table 4). The brown stripe material was taken from the plots of the Experiment Station at Baraguá, Cuba, where the eye spot does not exist at the present time. The eye spot material was taken from the experimental plots in western Camagüey Province upon plants badly diseased with eye spot but with very little brown stripe.

TABLE 4.—Average sizes of 100 spots of brown stripe and eye spot on mature leaves of *D 109* and *F. C. 306* canes

Cane variety	Disease	Total length in mm.		Width in mm.		Length-width ratio of spot
		Spot and halo	Spot	Spot	Halo	
F. C. 306	Brown stripe	25.0	22.9	1.16	1.05	20 to 1
	Eye spot	16.7	8.9	1.79	3.90	4.97 to 1
D 109	Brown stripe	21.4	19.5	1.00	0.95	20 to 1
	Eye spot	19.8	8.8	2.40	5.50	3.67 to 1

From table 4 it is clear that there is a very striking difference in the shape of the spots on these two varieties, the eye spot being about $1/4$ as wide as it is long, while the brown stripe is about $1/20$ as wide as long in typical cases. In the beginning stages these differences are not so great, but on mature leaves typical spots are about as above. The measurements of the width of the halo in the eye spot did not consider the long bands extending from the spots in advanced cases on these two varieties of cane. These bands are characteristic of the eye spot and have never been seen on any variety infected only with brown stripe. The width of halo with these two diseases varies with varieties, but so far as observed is usually greater in the case of the eye spot disease. A further marked difference is in the appearance of the actual spots themselves. The eye spot is not only of a brighter Bordeaux red color, but young spots have a stippled appearance rather than a solid color. Furthermore, the borders of the larger spots have this stippled appearance rather than the solid color of brown stripe. Also the halo surrounding the eye spot is from four to five times as wide as that around the brown stripe. These symptoms are so characteristic of the respective diseases that a little study in the field enables one to determine readily which disease is under observation.

The stripes, in the case of brown stripe, increase in size on many varieties, until they become 8–10 cm. long and up to $1/2$ cm. in width. It is in these larger elongated spots that the fruits of the fungus appear in greatest abundance. The species causing brown stripe sporulates very sparsely as compared with the eye spot *Helminthosporium*, and it is with considerable difficulty that the spores are secured in any quantities from diseased leaves in the field. While sporulation may occur on the stripes of affected leaves in moist chambers, great care must be used to secure the maximum number of mature spores when measuring for identification. In the culture jars described, the brown stripes become thoroughly covered with the spores, and as the leaves die (without contamination in the aseptic

jars), the infected areas become densely covered with conidiophores and spores. The measurements taken for both the eye spot and brown stripe

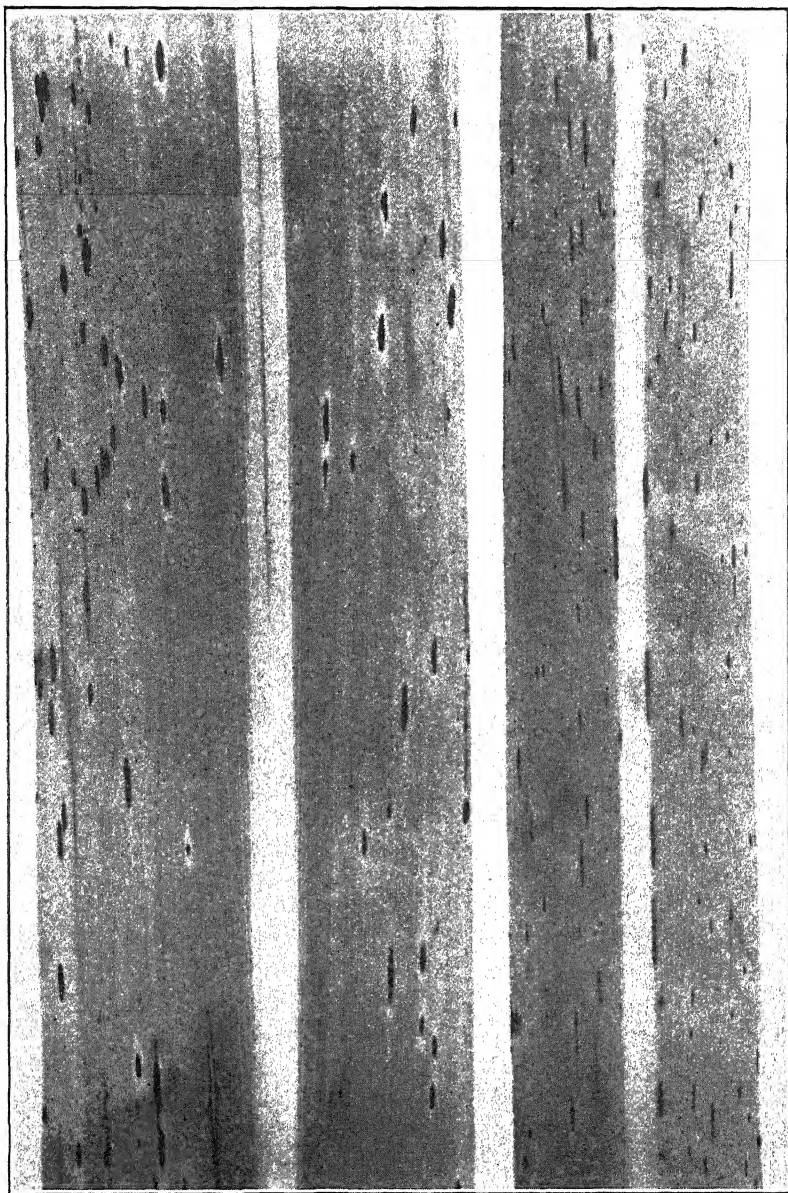


FIG. 4. Left—Typical eye spot on D 109 cane leaf. Right—Typical brown stripe on Cristalina cane leaf.

species of *Helminthosporium* were secured from material taken from these culture jars. Contrary to results secured from field collections the average measurements of spores from the jars were remarkably uniform, varying less than $3\ \mu$ in length from the general average given and less than $\frac{1}{2}\ \mu$ in width when several lots of 100 or more spores were measured.

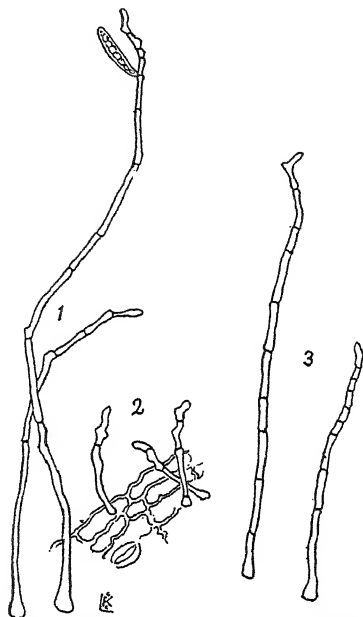


FIG. 5. Camera lucida sketch showing relative size of typical conidiophores of three cane *Helminthosporiums*.

- 1—Conidiophores of *Helminthosporium ocellum* n. sp.
- 2—Conidiophores of *H. sacchari* Butler.
- 3—Conidiophores of *H. stenospilum* Drechsler.

Under such conditions the spores of the *Helminthosporium* causing brown stripe are of a dark olivaceous color with a thick peripheral wall which is considerably thickened on the convex side. In my measurements of more than 1,000 spores produced in the glass cylinders previously described, the range of spore length was from 54 to $131.6\ \mu$ and of spore width from 11.3 to $18.8\ \mu$ with weighted averages of $89.6\ \mu$ and $15\ \mu$ respectively. These measurements are slightly greater than those given by Drechsler (9) for the brown stripe organism, which he has named *H. stenospilum*.

Varietal Susceptibility of Sugar Cane to the Brown Stripe Disease

Table 5 indicates the susceptibility of the principal cane varieties to this disease when grown under field conditions at Central Baraguá, Cuba.

TABLE 5.—*Relative susceptibility of cane varieties to brown stripe in Cuba*

GROUP I.—Varieties which are very susceptible. The new leaves become thoroughly covered with stripes and elongated spots.

Cristalina	B H 10 (12)
Caña Blanca	Morada (La. Purple)
B 3696	P R 16/6
B 3405	POJ 2221

GROUP II.—Varieties less susceptible than Group I.

Badila	C 291
S C 12/4	C 483
B 1809	D 109
B 6450	D-1135
P R 719	F C 306
D 216	Kassoer
C 555	B 3412
D 74	D 116
D 117	

GROUP III.—Varieties with moderate infection upon the older leaves.

Java Unknown	P R 712
H 109	P R 724
FC 137	B 4596
FC 214	Caña Criolla
POJ 2725	Negrita
P R 16/13	C 35
P R 209	POJ 36
P R 701	B 208
P R 704	C 519
P R 707	POJ 2714
P R 709	Bambo Blanca
B 109	Bambo Morada
C 291	C 553
Yellow Caledonia	D 99
	D 108

GROUP IV.—Varieties slightly susceptible or resistant.

Kavangire	Cayana 10	T 507
D 433	M 36	Uba
C 390	POJ 234	Cavangire
Tjepering	POJ 826	C 64/21
Co 210	POJ 979	C 653
Co 213	POJ 2379	Penang
Co 214	POJ 2727	
Co 232	Burra	
Co 281	SPI 33243	
POJ 228	Merthi	
	Oshima	
	Teckcha	
	T 439	

Field Losses from Brown Stripe

Brown stripe, while it would not be considered a major cane disease, as is the eye spot, is one of the most destructive of the minor cane diseases in Cuba. It becomes destructive during periods of dry weather when the vitality of the cane is lowered, and at times the stripes are so profuse as thoroughly to cover the leaves of the more susceptible varieties. Under such conditions the disease hastens the death and drying of the cane leaves. Vigorously growing cane suffers slight injury, and the only control measures justified at present are such cultural operations as may economically be carried out to maintain vigorous growth of the plants.

SUMMARY

1. The eye spot disease of sugar cane is widely distributed in Cuba, where it is destructive upon certain varieties of cane.

2. This disease, previously attributed to *Helminthosporium sacchari* Butl., does not agree in symptoms with the Helminthosporiose described and figured by Butler and Hafiz.

3. The organism causing eye spot is a *Helminthosporium* but differs from *H. sacchari* Butler and *H. stenospilum* Dreschler in color and length of spores and sporophores, as well as in the type of lesions produced upon the host plant.

4. The eye spot organism is provisionally named *Helminthosporium ocellum*.

5. A third *Helminthosporium* disease of sugar cane, brown stripe, has been found. Proof of parasitism of the causal organism, *H. stenospilum*, has been made by pot cultures and inoculation of plants growing under aseptic conditions.

6. A method for growing and inoculating plants under aseptic conditions has been worked out, which, it is believed, will enable a comparison of parasitic fungi under much more uniform conditions than is secured from field collections or the usual moist chamber methods.

7. Cane varieties differ greatly in their susceptibility to the brown stripe and eye spot *Helminthosporiums*, both of which are widely distributed in Cuba.

TROPICAL PLANT RESEARCH FOUNDATION

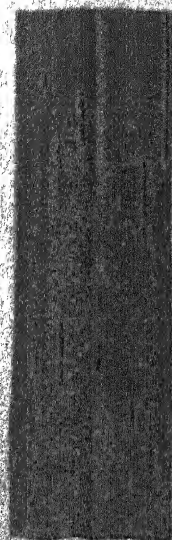
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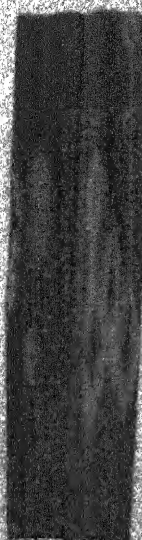
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EXPLANATION OF PLATE XV

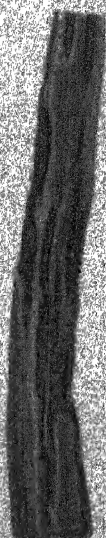
- I. Early stage of brown stripe disease upon Cristalina cane.
- II. Brown stripe in a late stage of the disease upon Cristalina cane.
- III. Early stage of eye spot upon D 109 cane.
- IV. Late stage of eye spot disease upon young B 3412 cane. The original spots enlarge to form a blotch.
- V. Eye spot in a late stage of the disease upon D 109 cane.
- VI. Copy of helminthosporiose of cane leaf—as shown by Butler and Hafiz on their Plate I, fig. 3 (4).
- VII. Typical spores of average size of three parasitic *Helminthosporium* species on cane drawn to the same scale.
 1. Spore of *Helminthosporium ocellum* n. sp., causing the eye spot disease of cane.
 2. Spore of *Helminthosporium sacchari* Butler, causing the Helminthosporiose disease of cane.
 3. Spore of *Helminthosporium stenospilum* Drechsler, causing the brown stripe disease of cane.



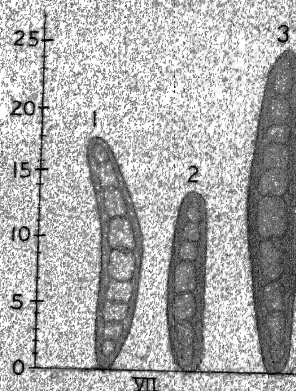
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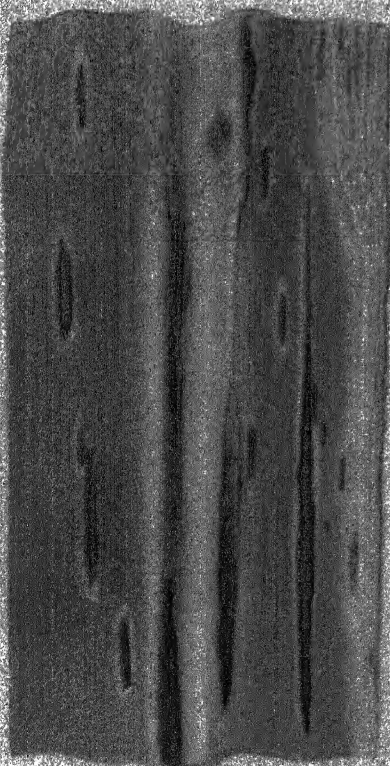
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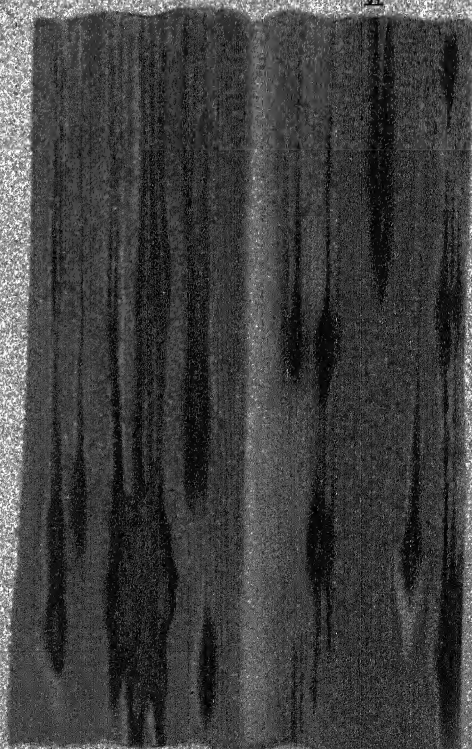
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VI



II



V

THE EFFECT OF A MANGANESE DEFICIENCY ON THE SUGAR CANE PLANT AND ITS RELATIONSHIP TO PAHALA BLIGHT OF SUGAR CANE

H. ATHERTON LEE AND J. S. MCHARGUE

INTRODUCTION

Pahala blight is a disease of sugar cane which takes its name from the locality where it was first observed and most commonly occurs, Pahala, a small town towards the southern extremity of the island of Hawaii, of the Hawaiian group. There is very little literature concerning this disease; Cobb (1) first described the disease in 1906, ascribing it to the leaf-splitting fungus, *Mycosphaerella striatiformans*. Cobb (2) also published fine colored illustrations of the leaf symptoms of the disease in 1909.

Since then the disease has been observed recently at Olaa on the island of Hawaii, and at Kilauea on the island of Kauai; but other than these instances in the Hawaiian Islands, there is no knowledge of the occurrence of this disease of sugar cane in other countries.

DESCRIPTION OF PAHALA BLIGHT

Pahala blight is not well named from the pathologist's viewpoint, for the disease is not a blight but is more properly a partial chlorosis: the leaves lose their chlorophyll in long, white streaks. There are many different degrees of severity of Pahala blight symptoms, but the most commonly occurring symptoms are the presence on the leaves of these long, whitish chlorotic streaks which are confined to the leaf blades and do not extend down into the leaf sheaths (Fig. 1). It is usually the third, fourth and fifth leaves from the youngest unfolded leaf of the cane top which show the symptoms most noticeably. These streaks may occupy different proportions of the leaf area, depending on the severity of the disease. As the disease continues, small reddish spots occur along the length of the white streaks, due to the killing of the white tissues. On these red spots the fungus *Mycosphaerella striatiformans*, found by Cobb and at the time thought to be the cause of the disease, is frequently found. Plants affected to such a degree that red spots begin to appear are usually considerably stunted by the disease. The red spots may become so numerous as to cause continuous reddish-brown streaks. In advanced cases the leaves of blight-

affected plants split along the reddened streaks (Fig. 2), and at one time blight was known to some extent as the leaf-splitting disease. Pahala blight of sugar cane in our experience has always occurred in soils of a neutral or alkaline reaction.

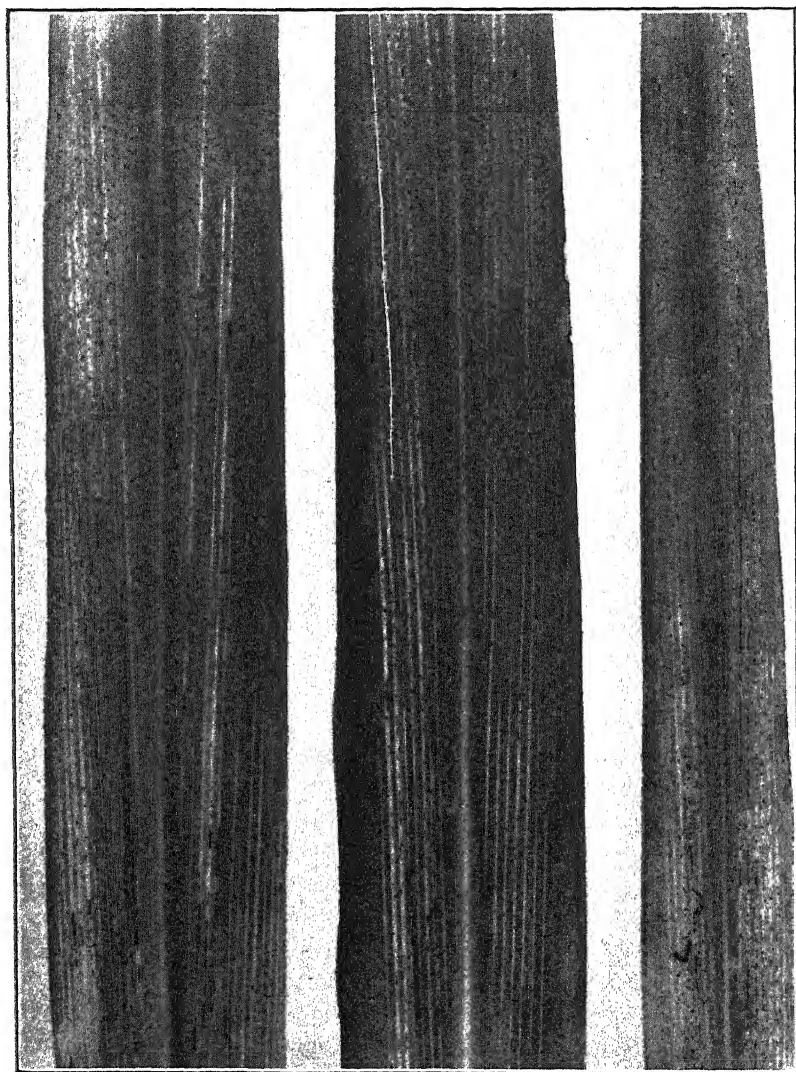


FIG. 1. Pahala blight symptoms on leaves of Badila variety of sugar cane, showing chlorotic streaks with red spots beginning to appear.

DISEASES WITH WHICH PAHALA BLIGHT MIGHT BE CONFUSED

In sugar cane there are leaf mutations with white streaks sometimes called sports, similar to those found in corn, which by one unfamiliar with these mutations might be confused with blight. However, in the case of such white leaf sports, the edges of the streaks are very definite and clearly defined; moreover, in the case of these sports there are usually but one or two streaks, at most five or six, while in the case of Pahala blight the longitudinal streaks are usually narrower but much more frequent on a single leaf. A sport, moreover, may usually be identified by finding the white streaks in a definite given position on succeeding leaves on the same side of the cane stalk, a distribution which would not apply to the streaks of Pahala blight.

Leaf scald, a disease of sugar cane in Australia, Fiji, Java, and the Philippines, in its early stages also causes a white longitudinal streak. Such a streak frequently runs down on to the leaf sheath, which is a quite distinct character from the chlorotic streaks of Pahala blight which are mostly confined to the leaf blades; the streaks of leaf scald are also wider, and not so numerous on a single leaf. The leaf streak disease of sugar cane in Natal, Egypt, and Mauritius might be confusing in the literature but should be easily distinguished by its yellowish streaks rather than the chlorotic whitish streaks typical of blight.

Pahala blight might also be confused with limestone chlorosis and other forms of chlorosis in which iron is deficient. In these iron-deficiency forms of chlorosis the whole leaf turns a whitish or whitish yellow color; in such types of chlorosis the streaking is not so definite as in the case of Pahala blight, and there is usually not the stunting effect on the plant as in the case of Pahala blight.

PREVIOUS EXPERIMENTS ON PAHALA BLIGHT

Some experiments with Pahala blight of sugar cane were described by Williams (7) in 1920, in which he was able to avoid the disease in a field in which blight usually occurred by (1) excavating a plot from the affected field and substituting a volcanic mud-flow soil from a field in which blighted plants had never been found; and (2) incorporating a large proportion of organic matter (stable manure mixed with sugar-cane press cake and bagasse) in plots with the usual field soil. In plots where a layer of charcoal was placed under the usual field soil, for the purpose of absorbing possible toxic gases arising through the soil, the disease reoccurred without abatement. Williams concluded (1) that the cause of Pahala blight resided in the soil, (2) was of a character other than toxic gases, and (3) could be corrected by adding organic matter to the soil.

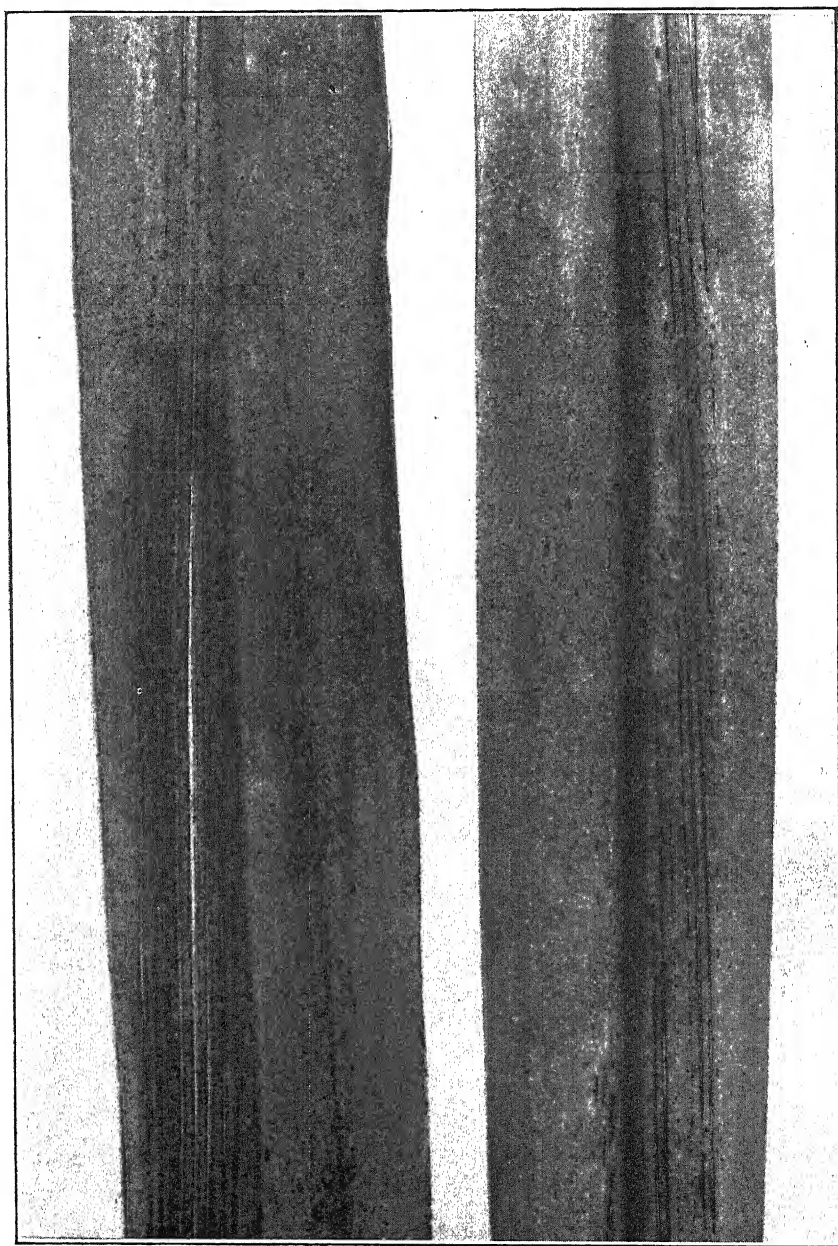


FIG. 2. Pahala blight symptoms on leaves of Badila variety of sugar cane, showing red streaks following chlorotic streaks, and the splitting of the leaves along such streaks.

In 1925 McGeorge (3) showed both experimentally and in field tests that applications of sulphur to the soil at the rate of from 1 to 2 tons an acre resulted in an entire recovery of the cane from Pahala blight in from 4 to 6 months after the treatment. He concluded that Pahala blight is a physiological or nutritional disturbance induced by soil conditions. McGeorge presented some analyses of blighted leaves as contrasted with normal leaves and it is interesting to note that there is no correlation between the presence or absence of iron in the leaves and the presence or absence of blight.

FIELD EXPERIMENTS WITH MANGANESE

McLean and Gilbert (5), in Rhode Island, had just shown that manganese solutions applied to the foliage of chlorotic spinach would bring about a quick recovery from the chlorosis, and the senior author (Lee), following their work, was experimenting with iron and manganese in relation to Pahala blight. Manganous sulphate and iron (ferrous) sulphate in solutions and as dry dusts were applied to the cane foliage of plants affected with the blight. The manganous sulphate brought about a prompt recovery of the cane, while the iron sulphate burned the leaves. This led to a more complete experiment in which manganous sulphate crystals were powdered and mixed with fungicidal dusting sulphur, the latter serving merely as a carrier, and applied to the foliage. Three different combinations of manganous sulphate, in sulphur as a carrier, were tried as contrasted with iron sulphate also in a sulphur carrier: there were four replications of the plots of each treatment, each plot consisting of four rows of cane, each row about 60 feet long. The manganous sulphate and iron sulphate crystals were not dehydrated but were merely crushed and ground fine before being added to and mixed with the sulphur carriers. The response to the successful treatments was noticeable five days after application and increased to a very considerable degree of recovery in three weeks. A description of the results of these treatments is contained in a letter of November 4, 1925, from Mr. J. C. Thompson, Research Agriculturist for the Hawaiian Agricultural Company, at Pahala, Hawaii, which is quoted as follows.

"In view of your request for a report on your dust experiments in Fields Middle Naahala and Lower Aliona, put in on October 16 in connection with the Pahala blight investigation, I beg herewith to report as follows:

First Series of Replications

Check Plot: Not very badly blighted, but blight found evenly distributed through the four lines of cane in the plot.

Dust No. 13:

20% Iron sulphate: Apparently no result from present appearances; dusted plot same as check plot.

Dust No. 14:

20% Manganous sulphate: Cane almost fully recovered. Good dark green color as compared to light green of check plot. Manganous sulphate action on blighted leaves found, that is, the characteristic well pronounced dark green-colored stripes and the yellowish white diseased stripes.

Dust No. 15:

30% Manganous sulphate: Practically identical results as with Dust No. 14.

Dust No. 16:

20% Manganous sulphate plus 15% ammonium sulphate: Almost fully recovered with the stand a dark green color as compared to the lighter green check plot.

Second Series of Replications

Check Plot: Cane stand consisting of four lines of cane very badly blighted and all yellow.

Dust No. 13:

20% Iron sulphate: No apparent difference between dusted plot and check plot.

Dust No. 14:

20% Manganous sulphate: Fair recovery although not so pronounced as to be noticed immediately. Manganous sulphate characteristic throughout when compared with check plot.

Dust No. 15:

30% Manganous sulphate: A better recovery than with Dust No. 14.

Dust No. 16:

Manganous sulphate, 20%; ammonium sulphate, 15%: Best recovery as far as this series is concerned, although not very pronounced. Sufficient difference between this plot and check plot, but no total recovery.

Third Series of Replications

Check Plot: Four lines blighted throughout.

Dust No. 13:

20% Iron sulphate: No apparent difference between this plot and check plot.

Dust No. 14:

20% Manganous sulphate: Fairly good green stand. Some blighted leaves but fairly dark green throughout as compared to the yellow blighty stand of the check plot.

Dust No. 15:

30% Manganous sulphate: Almost total recovery from blight. Good dark green healthy color except for the lighter-green mosaic leaves. Check plot light yellow and blighty color.

Dust No. 16:

Manganous sulphate, 20%; ammonium sulphate, 15%: Dark green and almost total recovery of shoots except for mosaic and a few blighted leaves with characteristic manganous sulphate tinge. Check plot yellow and blighted.

Fourth Series of Replications

Check Plot: Fairly good stand although it still retains its yellow tinge.

Dust No. 13:

20% Iron sulphate: No apparent action on blight. Leaves of whole plot badly burnt, more especially in case of diseased leaves. Healthy leaves also burnt.

Dust No. 14:

20% Manganous sulphate: Dark green stand good recovery with slight blight left. Manganous characteristic on leaves. New leaves healthy. Same for both plots; check plots light green with blighty tinge.

Dust No. 15:

30% Manganous sulphate:

(a) Fair recovery but not so noticeable due to mosaic disease in the plot which gives a rather light greenish color. Poor stand in this plot. Manganous sulphate characteristic of leaves present. Check plot fairly blighted.

(b) This plot showed fairly good recovery with fairly good green color to leaves. Few blighted leaves. Slight amount of mosaic disease present. Check plot only slightly blighted, with mosaic present also.

Dust No. 16:

20% Manganous sulphate; 15% ammonium sulphate: Good recovery. Some wholly recovered. New leaves healthy. Dark green color characteristic of manganese-treated leaves very pronounced. Check plot, yellow and blighty."

To summarize the results: iron sulphate resulted in no indications of recovery by the cane; on the contrary, iron sulphate resulted in a burning of the foliage of cane plants affected by blight. On the other hand manganous sulphate in a 20 per cent mixture in sulphur brought about a considerable recovery of the cane from blight. The mixture of 30 per cent manganous sulphate in sulphur seemed to allow plants a better chance of recovery than the 20 per cent mixture. The mixture of 20 per cent manganous sulphate and 10 per cent ammonium sulphate in sulphur seemed to induce better recovery than any of the other mixtures.

In conversation, P. S. Burgess, now of the Arizona Agricultural Experiment Station and formerly of the Experiment Station of the Hawaiian Sugar Planters' Association in Honolulu, stated that he tried out iron sulphate treatments against Pahala blight of sugar cane, with negative results similar to those just recorded.

Although it is a common impression that mineral elements are not absorbed through the cuticle by the leaves of plants, yet there has been at times some evidence in California (Ballard) that nitrogen from nitrate of soda was absorbed by the leaves of apple trees when applied as liquid spray. Certainly the present results indicate that manganese and possibly nitrogen from ammonium sulphate are absorbed and utilized by the leaves when applied to the foliage of sugar cane.

Subsequent to these results at Pahala, a considerable recovery from Pahala blight at the Kilauea Sugar Company was secured with applications of 30 per cent powdered manganous sulphate crystals in a sulphur carrier as shown in a letter of August 19, 1926, from Mr. Royden Bryan, Assistant Agriculturist at the Experiment Station of the Hawaiian Sugar Planters' Association, Kilauea, Kauai.

"Herewith submitted is a report of the effect of manganous sulphate against Pahala blight in Field No. 28, Kilauea Sugar Plantation Company.

The cane is the variety U. D. 1, ratoons, unirrigated. The plots, two treated, and two untreated as checks, each consists of 4 rows of cane; each row about 80 feet long. The treated plots were dusted with 30 per cent manganous sulphate in a sulphur carrier on July 2, 1926. Upon visiting the experiment on August 17, 1926, 45 days later, I observed a vast improvement in the treated plots. Although the cane in the checks showed a slight recovery from the blight, that in the treated plots had almost fully recovered, being ahead in growth and the new leaves free from Pahala blight.

Conclusion: U. D. 1 cane having Pahala blight showed an almost complete recovery from the blight, 45 days after being dusted with manganous sulphate."

From these field results, both at Pahala on the island of Hawaii and at Kilauea on the island of Kauai, there was a strong suggestion of relationship between the presence of manganese and the occurrence of Pahala blight. As stated previously, the occurrence of blight in the Hawaiian Islands has in all cases been in neutral or slightly alkaline soils. The working theory advanced was that sulphur applications to the soil, found by McGeorge to be so successful in curing Pahala blight, brought about a change in the soil reaction from neutral or slightly alkaline to acid and that in an acid soil complex, manganese was available to the plants, while in the original neutral or alkaline soil complex, manganese was not sufficiently available to the plants.

Experiments were therefore undertaken by the junior author (McHargue) to prove or disprove this theory that a manganese deficiency is the cause of Pahala blight of sugar cane.

CHEMICAL ANALYSES OF PAHALA BLIGHT LEAVES

First the presence or absence of iron and manganese in normal, partially affected, and severely affected Pahala blight plants was determined by analysis. The results of these analyses are shown in table 1.

These analyses showed that whereas there was no correlation between the iron content of leaves and the severity of Pahala blight, there was a direct correlation between the manganese content of such leaves and Pahala blight.

Previous work on manganese as a plant constituent had failed to show its essential relationship to plant growth. The junior author of this paper

[McHargue (4)], however, showed that many chemical reagents have manganese impurities, a factor which was not recognized in earlier experiments, and such impurities in supposedly manganese-free culture solutions nullified the value of the experiments. He showed in experiments with carefully purified chemicals in sand and solution cultures that manganese was, and is, an essential constituent of many plants.

TABLE 1.—*Analyses of normal, semi-chlorotic, and chlorotic sugar cane leaves*

Material present	Normal green leaves Per cent	Semi-chlorotic leaves Per cent	Chlorotic leaves Per cent
Ash (crude)	4.236	3.663	4.553
Iron (Fe)	0.036	0.016	0.024
Manganese (Mn)	0.003	0.0005	trace
Calcium (Ca)	0.224	0.333	0.380
Magnesium	0.232	0.146	0.154
Phosphorus (P)	0.155	0.173	0.220
Potassium (K)	1.455	1.441	1.648
Sodium (Na)	0.425	0.580	0.562
Sulphur (S)	0.161	0.267	0.240
Nitrogen (N)	1.305	1.650	1.690
Protein (N. \times 625)	8.156	10.313	10.560

In this sand culture work the symptoms of manganese deficiency in such plants as oats and wheat were strongly suggestive of the symptoms of Pahala blight of sugar cane. This led to the present experiments in which young plants of sugar cane were grown in manganese-free solutions to determine whether such an absence of manganese would cause the Pahala blight symptoms.

Vegetative cane cuttings of the Yellow Caledonia variety, a variety which is very susceptible to Pahala blight, were secured from the United States Department of Agriculture, Sugar Cane Breeding Station, at Canal Point, Florida, through the cooperation of Messrs. E. W. Brandes, R. D. Rands, and B. A. Bourne. These cuttings were planted in manganese-free sand cultures, germinated, and grown to a point where the cane shoots formed from the cutting had sufficient roots to be independent of the cane cuttings. The cuttings were then dug up and separated from the growing shoots with a knife; the growing shoots with their own roots were then planted in new manganese-free sand cultures. There were ten of such manganese-free cultures. The purpose of separating these cane cuttings was to eliminate any sources of manganese in the cuttings which might be available to the growing shoot and which would thus spoil the experiment with manganese-free cultures.

Ten cuttings were planted in sand cultures identical to the preceding except that manganese was included in the culture solution; the seed-piece cuttings were excised from the growing shoots in the same way and at the same time as the plants which were grown in the manganese-free cultures. All plants were then maintained under identical environmental conditions until the conclusion of the experiment.

The experiment was started in early September, 1926, in greenhouses at Lexington, Kentucky. The plants germinated, grew well, and were transplanted successfully, but as the winter months approached the plants did not grow in the greenhouse so well and vigorously as would have been the case in the open.

By February, 1927, all plants in the manganese-free cultures were showing the typical chlorotic leaf streaks and stunted appearance of Pahala blight. The control plants, with manganese present, under identical environmental conditions, grew a normal, luxuriant green foliage.

The experiment appears to prove rather definitely that Pahala blight of sugar cane is due to a deficiency of manganese in the available food supplies of the plant. The conclusions from these results with manganese-free sand cultures are supported by the recovery of sugar cane from Pahala blight when manganese was applied to the foliage.

DISCUSSION

A presentation of the subject would be incomplete if mention were not made of the results obtained by Schreiner and Dawson (6); they showed that a chlorosis of tomatoes occurring in Florida, on neutral or alkaline soils, was completely cured by additions of manganese sulphate to the soil. A recovery from the disease was also secured by adding stable manure to the soil. The leaf symptoms of their tomato chlorosis were identical with the Pahala blight symptoms of sugar cane. Apparently the two diseases are identical in nature.

The action of sulphur on the soil to increase the soil H-ion concentration and so make manganese in the soil complex more readily available to the plant, is in support of the experimental results recorded here.

There is also a relation of this nutritional disturbance to the use of ordinary fertilizer materials. A physiologically alkaline salt such as sodium nitrate, when used as a source of nitrogen, would considerably accentuate the disease, while the physiologically acid salt, ammonium sulphate, in itself, has been shown by plantation experience at Pahala to minimize the blight considerably. Acid superphosphate, which undoubtedly will have manganese impurities and would also be expected to have a slight tendency to increase the H-ion concentration of the soil, also has a slightly beneficial effect on Pahala blight in field practice.

Additions of organic matter to the soil probably alleviate Pahala blight not only by introducing small quantities of available manganese into the soil, but also by their action in increasing the soil H-ion concentration slightly and thus making some of the soil manganese more readily available.

It is apparent to the present writers that there are considerable differences among sugar cane varieties with respect to their demand for manganese. Yellow Caledonia, Yellow Bamboo, and possibly Badila, which are cane varieties very susceptible to Pahala blight, we would regard as active manganese feeders. On the other hand, resistant varieties such as D-1135 and possibly H-109 seem to have considerably less demand for manganese.

Since the name Pahala blight is not very appropriate from the viewpoint of plant pathologists, the more informative names, manganese deficiency, or manganese-deficiency chlorosis, are suggested as more accurate designations for this disease.

To the writers, one of the most interesting observations in connection with this work has been that what seemed abstract, or pure, research results obtained in one part of the world, *i.e.*, Kentucky, were so quickly utilized in an economic problem in Hawaii, in a rather distant part of the world. The writers feel that the results are also an outstanding evidence of the great value of cooperation.

SUMMARY

1. Applications of manganous sulphate to the leaves of sugar cane affected with Pahala blight brought about an alleviation of the disease and subsequent increased growth.

2. On analysis, leaves of sugar cane with severe Pahala blight symptoms showed only traces of manganese; leaves with slight symptoms of blight showed only slightly more manganese, while leaves of normal cane showed several times as much manganese. There is thus a correlation between the manganese content of leaves and Pahala blight.

3. There was no correlation between the iron content of sugar cane leaves and the presence or absence of Pahala blight symptoms.

4. Sugar cane plants grown in manganese-free sand cultures gradually developed Pahala blight symptoms until, at the end of six months, the disease was quite severe. Identical plants, except that they had adequate manganese, under identical environmental conditions, grew normally. From the results of this experiment the conclusion seems evident that Pahala blight results from a manganese deficiency. These experimental results and

conclusions are supported by the analytical results and the favorable response of the cane plant to manganese applications in the field.

EXPERIMENT STATION HAWAIIAN SUGAR PLANTERS' ASSOCIATION
AND
KENTUCKY AGRICULTURAL EXPERIMENT STATION.

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POWDERY MILDEW OF RASPBERRY

P. D. PETERSON AND H. W. JOHNSON

INTRODUCTION

Powdery mildew of raspberry was not reported in Minnesota until 1923 although it probably had been present prior to that time. It was common on both red and black varieties in that year (3). Since 1923 the disease has become more and more abundant, assuming epidemic proportions in plantings of the Latham variety throughout the state in 1925, 1926, and 1927. Damage, as indicated by stunting of the canes, has been confined to this variety of red raspberry. Local leaf infections of mildew have been found on other varieties both of red and black raspberries, also on blackberry and dewberry, but such infections are never abundant. The perithecial stage of the mildew has not been found in Minnesota on any of the varieties of raspberry and blackberry which are attacked.

A survey of the literature leads one to believe that the powdery mildew is not confined to Minnesota. A similar—perhaps the same—mildew has been reported on raspberries in Ohio, New York, Washington, Connecticut, Illinois, Maryland, Oregon, and Indiana. In 1920 an undetermined powdery mildew was reported on raspberries in Ohio (5). In 1922 Rankin (6) reported a similar powdery mildew in New York as the cause of cane dwarfing in several varieties of red and purple raspberries. He again called attention to this mildew in 1924 (7), stating that the Latham and Owasco varieties were most susceptible. He also observed infection on varieties of black raspberry. In 1926 Zeller (8) reported powdery mildew as the cause of severe injury to the Munger variety in Oregon. In 1927 the senior author observed powdery mildew on the Latham variety in Michigan and Wisconsin. The identity of the mildew has not been determined in any of these states. The ease with which the organism might be distributed on nursery stock, a point which will be brought out later in this paper, suggests the possibility that the mildew in most of these areas is the same as the mildew in Minnesota.

THE CAUSAL ORGANISM—ITS POSSIBLE IDENTITY

The occurrence of *Sphaerotheca humuli* (DC.) Burr. on several American species of *Rubus* is recorded by Burrill (1, p. 5) and by Salmon (4, p. 49). Burrill (1, p. 6) reports that this parasite does considerable damage

of surviving the winter in Minnesota is by means of mycelium within the buds.

In the fall of 1926 mildew-stunted plants of the Latham variety were dug and placed in trenches out-of-doors. On January 29 twenty-one of these

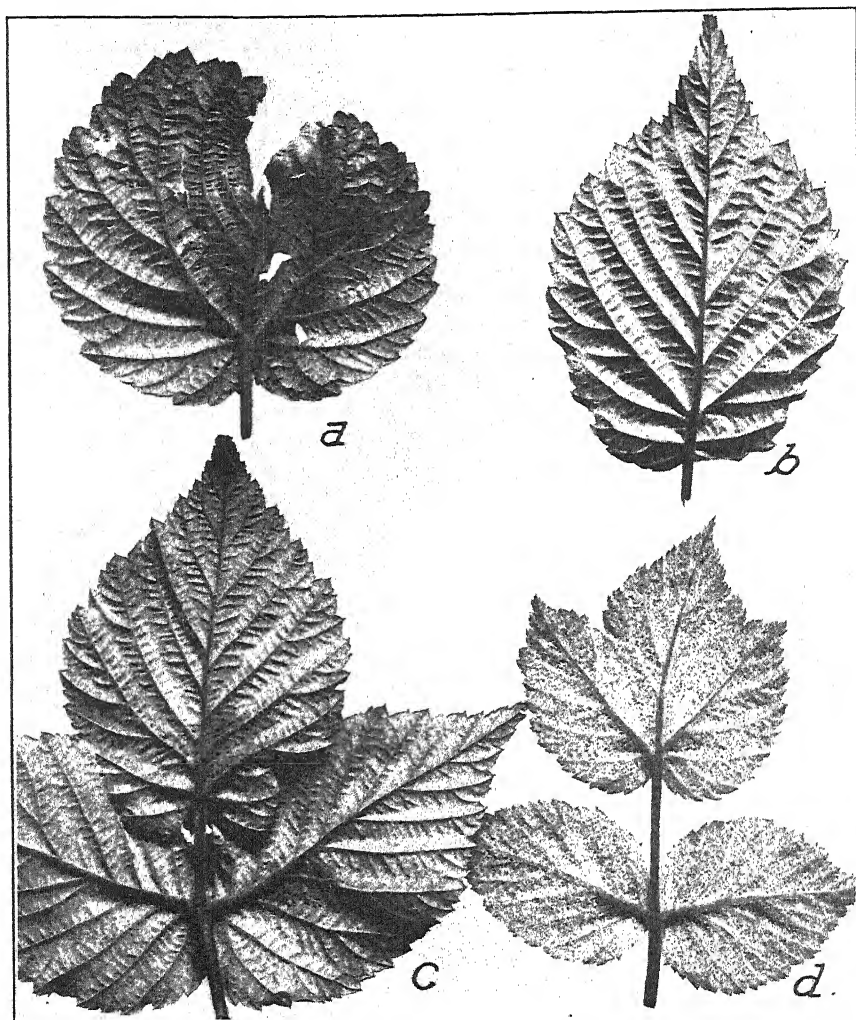


FIG. 2. Characteristic mildew infections on raspberry leaves. a. Old mildew lesion. Infected area devoid of leaf hairs. An area at the tip, killed by mildew, has fallen out. b. Numerous young local infections. Such infections produce leaf mottling similar to mosaic. c. Mildew, near base of leaflets, parasitized by *Cicinnobolus cesatii*. D. By. d. Stunted leaf from tip-infected area of the shoot.

plants were potted and placed in the greenhouse. Mildew developed on 11 of the 21 plants soon after the buds had opened. It developed only on shoots from buds in the stunted tip area of the canes. Infected shoots were entirely overrun by the mildew, which kept pace with the growing point and caused marked hypoplasia of the entire shoot. Such shoots usually died before reaching a length of four inches. They never bore fruit. Unaffected shoots under similar conditions averaged about one foot in length and produced several undersized berries.

On March 4, twenty-five additional plants were taken from the trench, potted, and placed in another greenhouse in which raspberries had not been grown previously. Mildew developed on shoots from buds near the tips of 6 of the 25 plants.

Experiments also were made to determine the percentage of mildew-infected canes in each of the several grades of commercial Latham red raspberry stock. Plants of three grades, Nos. 1, 2, and 3, were obtained from a local nursery. These plants had been cut back, according to the usual nursery practice, to a length of approximately 18 inches. The so-called No. 3 grade, which is not a commercial grade, consisted of plants too small to classify as No. 2 stock. Many mildew runts should be in this class. Judging from the evidence secured in our experiments that only those buds near the tips of the canes were infected, one would expect little or no mildew in the No. 1 stock because two or three feet of the canes had been cut off. Some mildew would be expected in the lighter No. 2 stock which had been cut back less severely. More canes with infected buds would be expected in the No. 3 stock. Mildew developed on none of the 50 No. 1 plants that were potted and placed in the greenhouse. On the other hand, 5 out of 50 No. 2 plants and 7 out of 50 No. 3 plants developed mildew when grown under similar conditions.

As a further check on the method of overwintering, the development of the disease was observed in the field in the spring of 1927. The mildew first appeared on shoots from near the tips of the larger canes and on shoots from buds at any position on the small, badly stunted plants. The first infections of this type were observed May 14, on shoots less than two inches in length. At this time the shoots were entirely overrun by the mildew. Secondary leaf infections were not observed until June 6, more than three weeks later.

It is clear from field studies that infected buds are located chiefly on areas of the canes which have been overrun with mildew the previous season. A few cases were found in which mildew developed from buds on the basal portion of large canes. Such infections can not be accounted for on the basis of tip infection. In these cases the buds were always located near the

base of very large canes which either had sent out lateral branches or showed a tendency to do so. It would seem that such buds might be stimulated to partial growth during the late summer, become infected, and then, due to a change in environmental conditions, again become dormant. Studies made during the summer of 1927 support this hypothesis. Several such buds were found covered with a sporulating growth of mildew. These buds stood well out from the cane, as contrasted to the snugly adhering dormant buds; and the bud scales were partly open, although no terminal growth was evident. Observations made later showed that these buds apparently resumed dormancy.

While the evidence from the above experiments is entirely observational, it leaves little doubt of the fact that the mycelium overwinters in the buds. Based upon similar evidence, mycelial overwintering has long been suggested in the case of a number of powdery mildews, including *Sphaerotheca pannosa*, *Uncinula necator*, *Podosphaera oxyacanthae*, and *P. leucotricha*. Recent work by Woodward (9) on the mode of perennation of *P. leucotricha* has proved definitely that this mildew overwinters by means of hyphae and haustoria within dormant apple buds. The same condition probably exists in the powdery mildew of raspberry. Histological studies are now in progress to ascertain more definitely whether this is true. It would appear from the evidence available that bud perennation may be a common method of overwintering in those powdery mildews that overrun terminal growth. The practical significance of this method of overwintering lies in the tremendous advantage gained by the pathogene through the early production of relatively enormous amounts of inoculum at a time when the host is particularly susceptible, that is, when new growth is being most abundantly formed.

SPRAYING AND DUSTING EXPERIMENTS

The serious epidemic of powdery mildew in Latham plantings throughout Minnesota in 1925 resulted in numerous requests for information regarding control. Spraying and dusting experiments were planned for the 1926 season, therefore, to secure data on the effectiveness of various fungicides in controlling the mildew. The manner in which the fungus overwintered was not known at this time, so no attempt was made to reduce the amount of inoculum by cultural methods.

The spraying and dusting experiments were made in a 25-acre propagative planting of the Latham variety which was known to have been heavily mildewed in 1925. All of the sprayed and dusted plots received a delayed dormant wash of 1-10 lime sulfur. This was followed in one plot by nine applications of 4-4-50 bordeaux mixture, in another by nine applications

of 1-40 lime sulfur, in a third by the same number of applications of copper-lime dust, and in a fourth by nine applications of sulfur dust. Both liquid sprays and dusts were applied at weekly or bi-weekly intervals, depending upon the weather conditions. The sprays were applied by means of a power sprayer which was fitted with three nozzles to the row and delivered the spray at a pressure of 200 pounds to the square inch. The dusts were applied with a power duster.

At the end of the season, the entire field was uniformly infected and stunted. The check plots were as good as, and, in a few cases, better than, the sprayed and dusted plots. Considerable foliage injury occurred in the lime sulfur plots from blossoming time until the end of the season. Prior to the blossoming period no injury was apparent. Sulfur dust caused a similar burning, but to a lesser degree. Hot and dry weather during most of the summer served to accentuate injury by the sulfur compounds. Neither bordeaux mixture nor copper-lime dust caused appreciable foliage injury.

CONTROL BY CULTURAL PRACTICES

Ten applications of fungicide having failed to control mildew in 1926, it was recognized that if spraying or dusting were to be successful, some method of reducing the amount of inoculum was necessary, especially in plantings of the wide-row propagative type. Field observations of the development of the disease during the 1926 season had indicated the probability of bud perennation, and the experiments discussed above were planned to ascertain whether this occurred. If this were the common method of overwintering, then eradication of the pathogene during the dormant season would be a practical and effective control measure. The practical application of this control measure requires adjustment to two sets of growing conditions: (1) those of propagative plantings, and (2) those of fruit plantings.

Mildew in Propagative Plantings

Mildew is much more destructive in wide-row plantings of the propagative type, owing to several factors: (1) the relatively slow growth of the canes because of plant competition, (2) the excellent opportunity for all tips to become infected because of the abundance of inoculum made possible by the matted rows, and (3) the poor air drainage within such rows. In such plantings few, if any, tips escape infection. Many are infected soon after they emerge from the soil. Such plants will usually grade No. 2 or lower, which means a direct economic loss to the grower. Of equal or greater importance to the grower is the ragged appearance of the planting.

Buyers have frequently turned from mosaic-free but badly mildewed plantings with the remark that they were not on a par with their own condemned stock. Plants in some such plantings average less than 2 feet in height at the end of the season in contrast to shoulder-high, mildew-free plantings of the same variety.

A method of eradicating mildew from plantings of this type was suggested by the practice of "clean-digging" already in use in some Minnesota nurseries. This practice is to dig all of the canes in the row each fall and to permit the new row to come up the following spring from underground parts in the inter-row space. Rows are thus alternated in the planting every other year; the cultivated inter-row space of one year becoming the row of the next year. Experiments were planned for the 1927 season to determine whether the practice of clean-digging could be adapted to mildew control.

The 25-acre field of Latham variety which had been used for the spraying and dusting experiments during the summer of 1926 was dug clean in the fall of that year, although no attempt was made to remove small, immature plants unfit for sale as propagative stock. It was assumed that most of these would winter-kill. On the contrary, however, many of them survived the winter, which was of average severity, and when growth started in the spring, shoots infected with mildew developed from these canes at close intervals over the entire field. In spite of this fact, the mildew developed more slowly in this planting than it had the previous season. This may have been due in part to weather conditions, but the eradication of a large number of infected buds in the digging of all mature canes was undoubtedly an important factor. However, as a demonstration of control this experiment was considered to be a failure.

In another part of the state, a second field was dug clean in the fall of 1926. All the canes suitable for sale as propagative stock were dug, and, in addition, a strip through the center of the field was harrowed until all of the young growth had been covered up or destroyed. In 1927, plants in the area which had been harrowed remained free from mildew, other than a few local infections, until late summer. The strips on both sides were abundantly infected early in the season. The plants in the harrowed area were also much freer from cane diseases, such as grey-bark (*Myco-sphaerella rubina*) and anthracnose (*Plectodiscella veneta*).

This experiment has demonstrated that powdery mildew can be controlled in wide-row propagative plantings, by the practice of clean-digging even though there are other infected plantings nearby. In such cases, spread from outside sources of inoculum is apparently so slow that very little stunting from mildew results.

Mildew in Fruit Plantings

Observations made during the past three seasons in plantings in which the Latham variety was being grown for fruit have shown that powdery mildew is not a serious problem in plantings in which either the narrow hedge or restricted hill system is used. In localities where severe stunting has occurred in wide-row propagative plantings, little or no stunting has been noted in fruit plantings in which the hedges have been narrowed down to a single row of plants or the hills restricted to from three to five canes. The canes in such plantings have maintained a growth level well above the height at which they are allowed to fruit. This permits sufficient pruning-back during the dormant season each year to insure removal of most, if not all, of the infected tips. In addition, it is necessary to remove and destroy all stunted plants and the late season growth which, as was shown in the authors' clean-digging experiments in propagative plantings, may live through the winter and serve as centers of infection the following spring.

SUMMARY

1. Destructive epidemics of powdery mildew have occurred in Latham red raspberry plantings throughout Minnesota during the past three seasons. The disease has been reported also from Ohio, New York, Washington, Connecticut, Illinois, Maryland, Oregon, Indiana, Michigan, and Wisconsin.

2. The perithecia of this powdery mildew have not been found in Minnesota, nor by recent observers in any of these other states so far as the writers have been able to determine. In the absence of the perfect stage, it has been impossible to identify the mildew definitely. However, Burrill and Salmon both record the occurrence of *Sphaerotheca humuli* on several American species of *Rubus*, and it is possible that this is the powdery mildew of raspberry discussed in the present paper.

3. Under field conditions in Minnesota, the mildew is invariably parasitized by *Cicinnobolus cesatii* D By. This may account for the failure to form perithecia.

4. The most striking symptom of the disease is the dwarfing of the leaves and the stunting of terminal growth from tip infection. Local infection of the leaves also occurs. In such cases the fungus is usually confined to the lower surface of the leaf but, under favorable environmental conditions, may spread over both leaf surfaces, the leaf petiole, and the stem. When local infections are numerous, the leaves assume a mottled appearance similar to that produced by mosaic.

5. Experiments have shown that the organism can persist throughout the winter in infected raspberry buds which, as a rule, are located in

the stunted-tip area of diseased canes. The apparent lack of perithecia and the fact that spore viability studies have shown that the conidia are very short-lived indicate that perennation within the buds is the common, and perhaps only, method the organism has of surviving the winter in Minnesota.

6. The disease was not satisfactorily controlled either by spraying or by dusting in large-scale field experiments made in 1926.

7. The practice of "clean-digging" has proved to be an effective method of eradicating the pathogene from propagative plantings of the Latham red raspberry in Minnesota. Growers following this practice eliminate almost entirely the stunting effect which results from early tip infections of powdery mildew.

8. Observations during three seasons have shown that powdery mildew is not a serious problem in fruit plantings in which either the narrow hedge or restricted hill system of culture is practiced.

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PRELIMINARY NOTE ON SNOWBERRY ANTHRACNOSE¹

M. F. BARRUS AND JAMES G. HORSFALL

The common snowberry, *Symphoricarpos albus* (L.) Blake, var. *laevigatus* (Fernald) Blake, in the vicinity of Ithaca, New York, is severely affected by an anthracnose. Numerous observations have revealed that severe defoliation and a destruction of the berries often accompany the disease. The name "anthracnose" is applied because of the resemblance of the causal fungus to that causing the familiar raspberry anthracnose.

The disease has been observed to be destructive in Delaware, Tioga, and Tompkins Counties in New York State, and the writers have received severely diseased specimens from Arkansas,² Iowa, and Wisconsin. Since apparently only a single note³ regarding the disease has been recorded in the literature, the publication of this paper appears warranted.

Snowberries are widely planted for the beauty of the snow-white fruits which persist after the leaves have fallen until well into the winter, but when plants are affected with anthracnose this character is tremendously impaired. Such losses are, of course, difficult to estimate, but they must be appreciable.

The disease appears on the leaves early in the spring as minute, dark-purple to black spots which enlarge slowly (Plate XVI). The center later becomes light- to dirty-gray in color with a margin of dark-purple to black. Spots are usually circular and sharply delimited from healthy leaf tissue, but their coalescence will often produce a large, ugly, irregular spot which is subject to cracking in the center. When marginal infections kill the tissues early, they cause marked hypoplastic deformations. Frequently the leaves are badly misshapen as a result of such infections. Inconspicuous lesions essentially like those on young leaves appear on flower-buds and leaf-buds before they unfold. Lesions were observed once on the petals of an open flower.

¹ The authors wish to express their indebtedness to Professor H. H. Whetzel, Department of Plant Pathology, Cornell University; to Dr. Anna E. Jenkins, Office of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture, for helpful assistance and criticism during the course of the investigation and for aid in reading the manuscript; and to Mr. W. R. Fisher for making the photographs.

² The specimen from Arkansas was secured through the kindness of Mr. William Horsfall, Fayetteville, Ark. Thanks are also due the Office of Mycology and Disease Survey, United States Department of Agriculture, for the contribution of the specimens from Iowa and Wisconsin.

³ Stewart, F. C. Anthracnose, *Gloeosporium* (?) sp. Notes on New York plant diseases, I. N. Y. (Geneva) Agr. Exp. Sta. Bul. 328: 393-394. 1910.

Small, dark-purple to black lesions can be found on the calyx end of fruits shortly after the petals fall (Plate XVII). These spots, which are fimbriately margined, do not appear to go much deeper than the cuticle and the first few layers of cells below. Berries, like leaves, infected when small, become lopsided. The spots appear as circular, somewhat sunken, pink lesions of various sizes on the more mature berries. Frequently an *Alternaria* invades the diseased tissue and causes a watersoaked lesion to develop which speedily involves the whole fruit. Such fruits soon become shriveled, dry, brown mummies. Occasionally late in the season, fruiting branches may be seen that are devoid of any berries except a few which emerged before the inoculum became copious.

In the early spring, the tender shoots often are attacked by the malady. Here the disease is expressed as small, oblong, and somewhat sunken spots having a dark-gray color. The lesions on the shoots are rarely, if ever, more than two millimeters in length.

Considerable difficulty in isolating the causal organism was experienced at first, before its identity had been established. In the early part of the investigation only an *Alternaria* and a *Gloeosporium* were isolated from diseased areas. Puncture inoculations with the *Alternaria* produced the watersoaked lesions described previously, but these were in no way comparable with anthracnose lesions. Atomizing the *Gloeosporium* conidia on to berries did not produce the disease. During the summer of 1926 an organism was isolated which was later identified as a species of *Sphaceloma* by Miss Anna E. Jenkins, who based her determination on gross cultural features. This fungus was obtained by spore dilution and from plantings of diseased tissue in tubes of agar. Two additional isolations of it were obtained in 1926, and during the season of 1927 it was obtained eight times from diseased leaves, twigs, and berries.

The causal organism is peculiar, for on standard culture media such as potato-dextrose agar it has almost none of the hyphal growth usually associated with fungi in culture. It grows very slowly, forming a tough mat of mycelium which appears characteristically folded and ridged on the surface with but little submerged growth. Superficially the growth looks like gelatine. An individual thallus will attain a size of one centimeter or so in diameter and then cease to grow, so that it has not been known to cover completely an agar slant. On potato or oat agar, the mycelium is predominantly red. In old cultures, the color may become dusky-slate-violet,⁴ but in more recent transfers the color is frequently light enough to be called vinaceous-buff. The color will usually diffuse from the mycelium

⁴ The color readings of cultures are based on RIDGWAY, R. Color standards and color nomenclature. Washington, D. C. 1912.

and impart a beautiful dark-red shade to the medium of cultures a month or so old.

Because spores were not available in pure culture at first, mycelium covered with moist cotton on young leaves in the greenhouse served as inoculum. In 10 days the minute infections were found. The fungus was reisolated 44 days after inoculation. Using the same technique, infection on young berries was observed in 6 days. The pathogene was successfully reisolated 41 days later. A third successful inoculation of snowberry leaves was accomplished later using spores subsequently produced in culture from the fungus reisolated from the first mycelial inoculation. In this case infection was apparent in 4 days. Plate XVI shows these leaves 39 days after the spores were sprayed on them. On this date the organism was reisolated and presented cultural characters indistinguishable from those originally observed.

Very little work upon control has been accomplished. An experiment in dusting bushes five times during the season of 1927 with copper lime dust, although inconclusive, gave some evidences of control. More attention to timing the applications should produce better results.

Cultures of *Sphaceloma fawcettii* Jenkins, *Sphaceloma ampelinum* DeBary, and *Plectodiscella veneta* Burkholder were secured from Miss A. E. Jenkins⁵ and compared simultaneously with the snowberry anthracnose fungus. These were found very similar, but nevertheless certain constant cultural characters, such as type of growth and color, indicated that the last is different from any of the others. Furthermore, none of these fungi infected young snowberry fruits, when at the same time this fungus did induce infection.

Because of the resemblance of the snowberry anthracnose fungus in culture to *Sphaceloma ampelinum* DeBary,⁶ the type species of the genus, it has been considered best to place it in the form genus *Sphaceloma*. Since no technical name appears to have been applied to the fungus, it is here described as a new species.

Sphaceloma symphoricarpi n. sp. Acervuli sub-cuticular to sub-epidermal becoming erumpent, solitary, gregarious, or confluent, irregular, from 50 to 300 μ in diameter, on leaves, fruits and stems, on leaves epiphyllous; stroma pseudo-parenchymatous above, prosenchymatous below; conidiophores at first hyaline then dark, unbranched, characteristically sharp-pointed, closely compacted, arising perpendicularly, often in tufts, from surface of stroma; conidia terminal, embedded in a gelatinous matrix, oblong-ellipsoid, $4.0 \times 8.0 \mu$ ($3.0-5.4 \times 6.0-9.0 \mu$) (observed in culture),

⁵ JENKINS, A. E. The citrus-scab fungus. *Phytopath.* 15: 99-104. 1925.

⁶ DEBARY, A. Ueber den sogenannten Brenner (Pech) der Reben. *Annal. der Oenol.* 4: 165-167. 1873.

typically bi-guttulate, one oil drop in each end, continuous, hyaline, later becoming slightly dark.

Habitat: Parasitic on *Symphoricarpos albus* (L.) Blake, var. *laevigatus* (Fernald) Blake; spots on leaves dirty-gray in center with dark-purple margin; on fruits at first dark-purplish to black with fimbriate margin, later formed spots becoming pink and sunken; on succulent stems small, oval, depressed lesions gray in center with dark margin.

Type locality: Forest Home, Ithaca, New York.

Distribution: New York, Wisconsin, Iowa, and Arkansas.

Material examined: Ithaca, N. Y., M. F. Barrus and J. G. Horsfall, C. U. Pl. Path. 15252 (type); Ithaca, N. Y., 1927, M. F. Barrus, C. U. Pl. Path. 15768; Ithaca, N. Y., 1927, J. G. Horsfall, C. U. Pl. Path. 16250, (artificial inoculation in greenhouse); Walton, N. Y., 1927, A. E. Jenkins, C. U. Pl. Path. 16305; Fayetteville, Ark., 1927, W. R. Horsfall, C. U. Pl. Path. 16306; Madison, Wis., 1927, R. E. Vaughan, C. U. Pl. Path. 16307; Ames, Ia., 1927, R. J. Haskell, C. U. Pl. Path. 16308.

These specimens, as indicated by their accession numbers (C. U. Pl. Path. No.), are to be found in the herbarium of the Plant Pathology Department of Cornell University, Ithaca, N. Y.

Authentic specimens have been deposited in the following herbaria: Pathological Collections, Bureau of Plant Industry, Washington, D. C.; New York Botanical Garden, New York, N. Y.; Royal Botanical Garden, Kew, Surrey, England.

SUMMARY

1. Snowberries are seriously affected with anthracnose in the vicinity of Ithaca, N. Y. The disease has been reported also from Wisconsin, Iowa, and Arkansas.

2. The symptoms of the disease are described, and Koch's rules of proof have been fulfilled for the causal organism.

3. The pathogene is described as a new species, *Sphaceloma symphoricarpi*.

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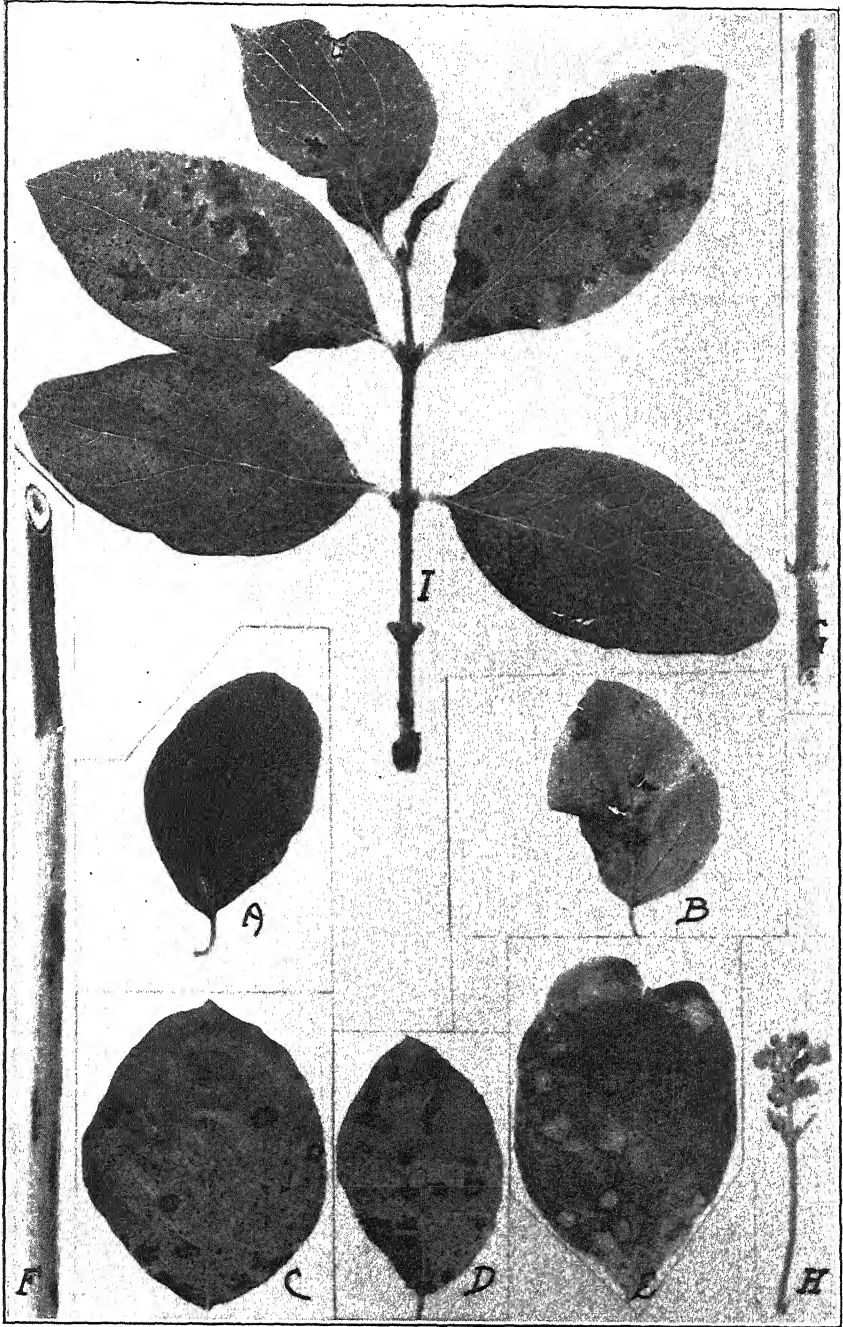
EXPLANATION OF PLATES

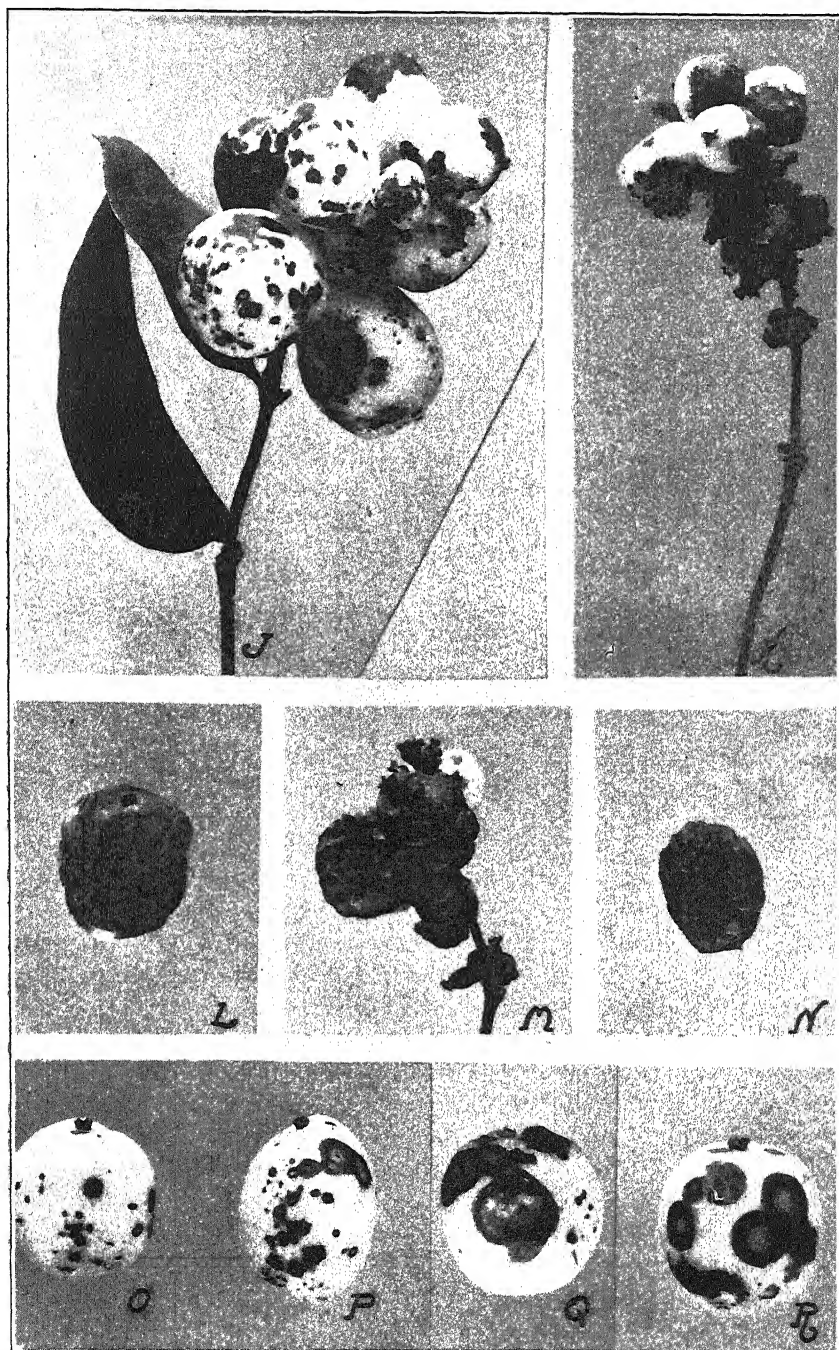
PLATE XVI

- A. Upper surface of young leaf of *Symphoricarpos albus* var. *laevigatus* showing lesions caused by *Sphaceloma symphoricarpi*.
B. Lower surface of young leaf showing lopsidedness caused by infection.
C. Lower surface of old diseased leaf.
D. Same as C, another leaf.
E. Upper surface of old diseased leaf. Note cracking above.
F. Small sunken lesions on stem.
G. Recent infections on succulent twig.
H. Inflorescence showing minute lesions on unopened flower buds.
I. Leaves 39 days after being inoculated with spores. Note the dead apical leaf and its deformed mate.

PLATE XVII

- J. Fruit cluster showing late-formed lesions.
K. Fruit cluster showing early fimbriate-margined infections. Note deformed berries.
L, M, N. Berries late in season mummifying because of *Alternaria* infection following *Sphaceloma*.
O, P, Q, R. Individual berries showing later infections. At Q, note minute black acervuli in the lesion.





THE DEMONSTRATION OF BACTERIA IN PLANT TISSUES BY MEANS OF THE GIEMSA STAIN¹

WILLIAM H. WRIGHT AND VLADIMIR SKORIC

The staining of bacteria in animal tissues has received so much more attention than those in plants that correspondingly better methods have been developed. In this respect the animal pathologist is more fortunate than the phytopathologist. It seems quite reasonable to suppose that some of the animal methods might be adapted for use in similar studies with plants.

A review of the literature shows that fuchsin has been the most generally used of the stains for bacteria in plant tissues. Dale (2) obtained good results using Diamond Fuchsin with light green as a counterstain. While making a study of the symbiotic bacteria in the leaves of the *Rubiaceae*, Faber (4) obtained variable success but found fuchsin as satisfactory as any stain tried.

The differential nature of the Gram stain offers much more of value for the study of bacteria in animal tissues than it does with plant tissues. Smith (14) recommends the Gram stain for the staining of bacteria in plant tissues when the bacteria are Gram positive. However, comparatively few bacteria pathogenic for plants are Gram positive.

Other stains, such as iron haematoxylin and Flemming's triple stain, have often given good results. Satisfactory preparations are sometimes obtained with water soluble nigrosin. Methyl violet has been found very satisfactory for the staining of *Bacillus tracheiphilus* in cucumber tissue. Preliminary treatment in tannin water is supposed to prevent excessive staining of the host tissue. Riker (10) used dilute carbol fuchsin to stain the bacteria of crown gall in plant tissue. Light green was used as a counter-stain. Magrou (8) successfully uses pyronine and methyl green on this material.

The variable results obtained when attempts are made to stain bacteria in plant tissue emphasize the need for more study of the staining processes for such material. The combining power of acid and basic dyes with pure proteins has been shown by Loeb (6). More recently Robbins (11) has made use of the same principle in the determination of the isoelectric range of plant tissue. Following this general procedure Naylor (9) developed a method of differential staining for sections or foot tips of various plants.

¹ Published with the approval of the Director of the Agricultural Experiment Station, University of Wisconsin.

By staining with acid and basic dyes of contrasting colors and washing with buffer mixtures at definite hydrogen ion concentrations, differences in the power of different parts of the cells to retain the dyes resulted in differential staining. The results of these investigators, as shown by the absorption of dyes, indicate that the isoelectric range for most plant tissues is between pH 4.6 and pH 5.0. These values are apparently much higher than those found for some of the bacteria causing plant diseases. In a recent study of the bacteria causing wilt of bean (*Bact. flaccumfaciens*), blight of bean (*Bact. phaseoli*), and pustule of soybean (*Bact. phaseoli sojense*), Sharp (13) found the isoelectric range of each of these organisms to be within pH 1.2 and pH 3.0.

If combination of the protein constituents of cells with dyes is as dependent upon the hydrogen ion concentration of the dye-protein mixtures as recent experiments seem to show, it should be possible, by selecting acid and basic dyes of contrasting colors, to differentiate bacteria in tissues from the tissue constituents.

THE GIEMSA STAIN

Giemsa's stain is a mixture or compound dye. Zoologists and animal pathologists have often used mechanical mixtures of dyes for differential staining. Aside from the contrasting colors due to the selective powers of the dyes in the mixtures, there may result, from the mixing new dye compounds with very valuable staining properties.

Romanovski (12) was one of the first to suggest the use of a mixture of acid and basic dyes for staining protozoa. The stain was later modified by Giemsa (5) and recommended for cytological and blood work. This stain was used by Dobell (3) in an extensive study of the cytology of bacteria.

The Giemsa stain is usually prepared from Azure I (Giemsa) containing a variable mixture of Azure A (asymmetric dimethyl-thionin) and Azure B (tri-methyl thionin). Azure II (Giemsa) is a mixture of Azure I (Giemsa) with methylene blue. These two dye mixtures are prepared for use in very pure methyl alcohol with eosin (tetrabrom fluorescein). Recently MacNeal (7) has developed a simple method of preparing methylene Azure A (asymmetric di-methyl-thionin) which may be used instead of the less stable methylene azure.

THE STAINING OF BACTERIA IN PLANT TISSUES WITH THE GIEMSA STAIN

Several staining methods were tried in the study of pathogenic bacteria in plant tissues and soybean nodule bacteria in root nodules of soybean. When compared with other methods, the Giemsa stain was found to give the best results. The purest grade of neutral methyl alcohol was used and very pure water added when dilution was necessary. A ready prepared,

dry form of the commercial dye was used to prepare a fresh solution each time. Several batches of the dye solution, when tested potentiometrically, were found to have pH values varying from 4.2 to 4.5. Sections gave the best staining results when treated with several changes of buffer solutions, near pH 6.0 for an hour or more. Reactions more alkaline tended to overstaining with the Giemsa stain. As a counterstain 2 per cent safranin in absolute alcohol was used.

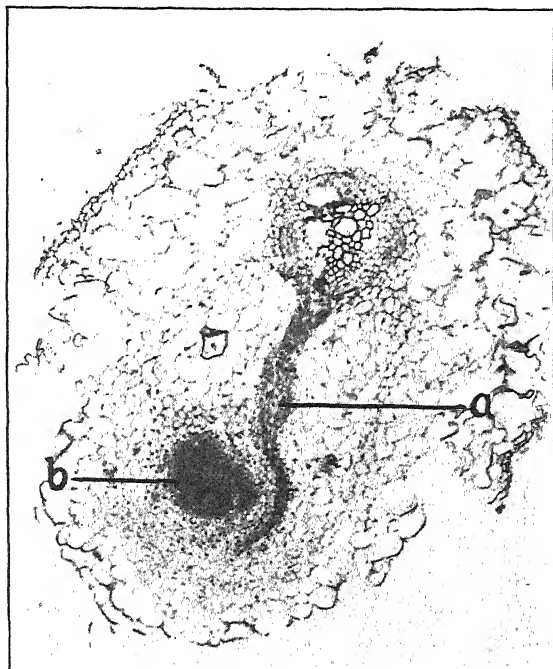


FIG. 1. Section of a soybean root nodule. The extension of the vascular structures from the root to the nodule may be seen at *a*. The cells showing dark at *b* are filled with deeply stained bacteria. Magnification 100 \times .

Tissues fixed by several methods were found to give very satisfactory results. The procedure of Bensley (1) as used for chondriosomes was used with good results.

Material embedded in paraffin was cut serially into sections of 4-6 micra in thickness. These, after the usual fixation and stretching, were treated as follows:

1. Remove paraffin in xylol.
2. Absolute, 80, 50, 30, and 15 per cent alcohol.
3. Wash in water and treat for an hour in several changes of buffer solutions near pH 6.0.

4. Stain in Giemsa stain for 10 to 15 minutes.
5. Wash in absolute alcohol until the tissues are nearly free from stain but the bacteria retain it. This varies with the pH of the material.
6. Dip the slides 3 to 6 times in the safranin solution.
7. Wash in absolute alcohol.
8. Xylol and then balsam.

The bacteria are deep blue, and, as in the case of the soybean nodule bacteria, the areas occupied by them can be seen with the naked eye. The photomicrograph of such a section is shown in figure 1. The general microscopic appearance of two sections is shown by the camera lucida drawings in Plate XVIII.

The bacteria are stained deep blue, plasma light blue, nuclei pink, nucleoli blue, and cell walls red. In cases where the tissue had undergone change, due to bacterial action, it was stained blue, but the bacteria were a deeper blue and sharply defined.

By means of this method, *Pseudomonas radicola* was stained in the root nodules of soybean, *Mycobacterium rubiacearum* in the leaves of *Psychotria bacteriophylla*, *Pseudomonas pisi* in pea, and *Pseudomonas phaseoli* in bean tissue.

SUMMARY

A method of staining bacteria in plant tissues is described, using Giemsa's stain with buffer solutions and safranin as a counterstain.

Four species of bacteria were stained, including two intracellular symbionts and two plant pathogens.

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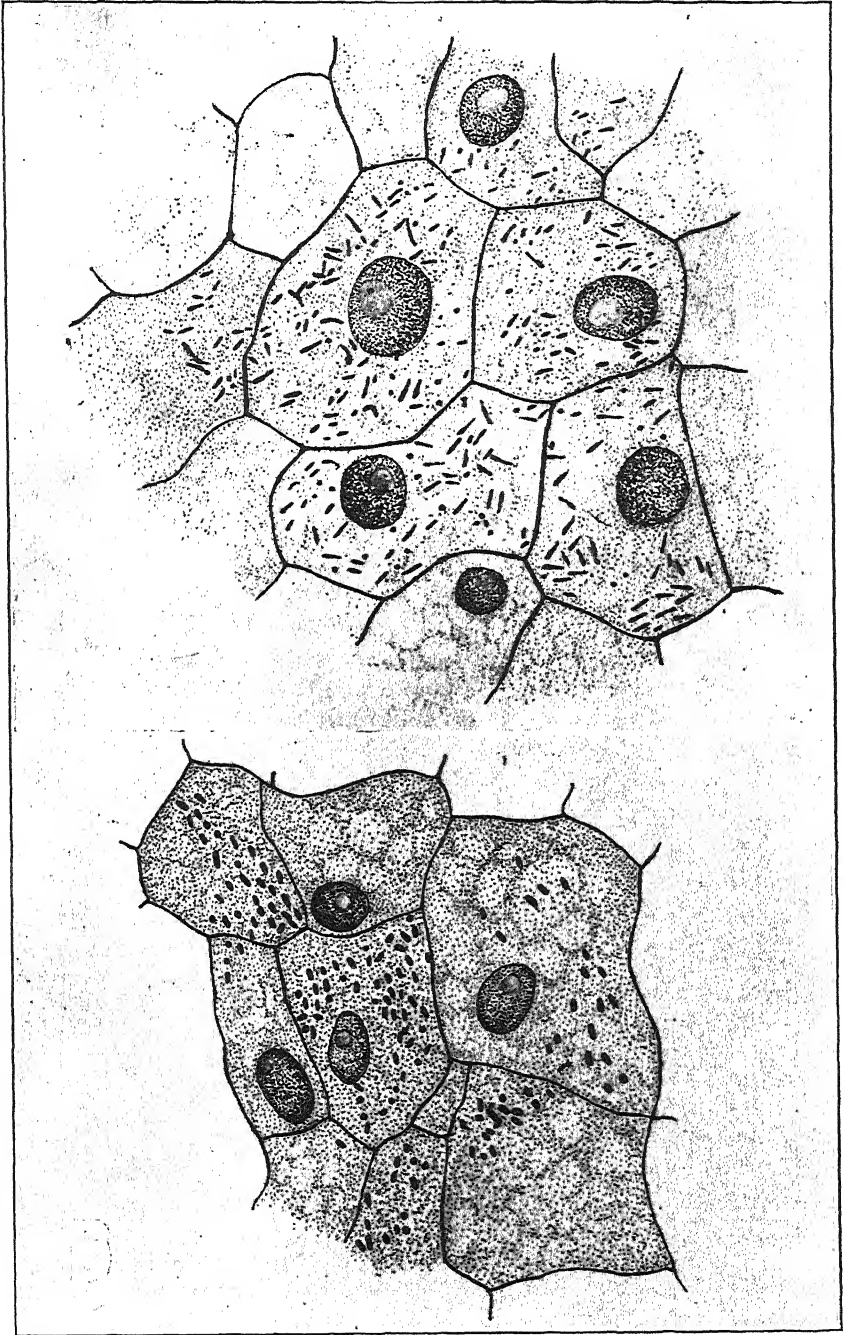
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EXPLANATION OF PLATE XVIII

A. Typical area in slightly infected part of a nodule of *Soja max*. Section cut 4 micra in thickness. Camera lucida drawing. Magnification 2000 ×.

B. Typical area in part of a nodule of a leaf of *Psychotria bacteriophylla*. Camera lucida drawing. Magnification 2000 ×.



STORAGE ROTS OF CRANBERRIES IN THE 1927 CROP¹

NEIL E. STEVENS AND HENRY F. BAIN

Continuing studies begun in the fall of 1926,² the writers have again attempted to measure quantitatively the actual storage rot caused by various fungi in cranberries from different growing regions. The results of the storage tests with the 1927 crop are given herewith. While for reasons that will be pointed out, the results are not strictly comparable in all respects with those of the 1926 tests, they furnish a fairly complete and accurate picture of the actual storage rot in the cranberry crop of the current year.

METHODS

The methods were substantially the same as in the previous test, fully described on page 650.² In presenting the results, however, a somewhat different method has been used, and the data for 1926 have been recalculated for comparison. The height of each column, figure 1, represents the total percentage of spoilage to January 1 in that particular lot of berries. Fractional parts of the total spoilage initiated by different causes were determined by means of cultures made at intervals of one month during the storage period, as described in the earlier paper. At the beginning of each two-weeks' storage test the percentage rot in the stock box was determined by actual count of representative samples of berries. Only sound berries were used for the test lot. In the present calculations, values for each test lot were corrected to represent the percentage of spoilage on a basis of total berries in the stock box rather than of sound berries in the test lot. The percentage values for an entire month were then determined by applying the values found in the two-weeks' test to the actual spoilage that occurred during the month, as determined by the difference between two consecutive counts of spoilage in the stock boxes. These monthly values were summed up to give the totals represented in figure 1.

RESULTS

The chief interest of these tests lies, of course, in the differences between 1926 and 1927. The results are summarized in table 1 and figure 1. In figure 1 the results are shown individually for the separate lots of berries, while in table 1 the lots are averaged.

¹ Investigation conducted cooperatively by the Office of Fruit Diseases, Bureau of Plant Industry, and the Wisconsin Department of Agriculture.

² STEVENS, N. E., and H. F. BAIN. Storage rots of cranberries in the 1926 crop. *Phytopath.* 17: 649-655. 1927.

Under the conditions of the tests, the Howes and McFarlin cranberries from Massachusetts, New Jersey, and Oregon showed somewhat more rot in 1927 than in 1926. The difference was greatest in berries from Massachusetts, and least in those from Oregon. Berries from Wisconsin, on the other hand, kept somewhat better in 1927 than in 1926. Examination of the graphs and of the first two columns of table 1 shows that the difference between 1926 and 1927 in Massachusetts and New Jersey is distributed among various causes as follows: There was a slight increase in sterile breakdown and in the loss from end rot; and there was a marked increase in the loss from *Guignardia* and *Glomerella*.

CAUSES OF THE DIFFERENCE IN LOSS IN 1926 AND 1927

After a careful study of the conditions of the experiment, the writers have been forced to the conclusion that the difference in results obtained

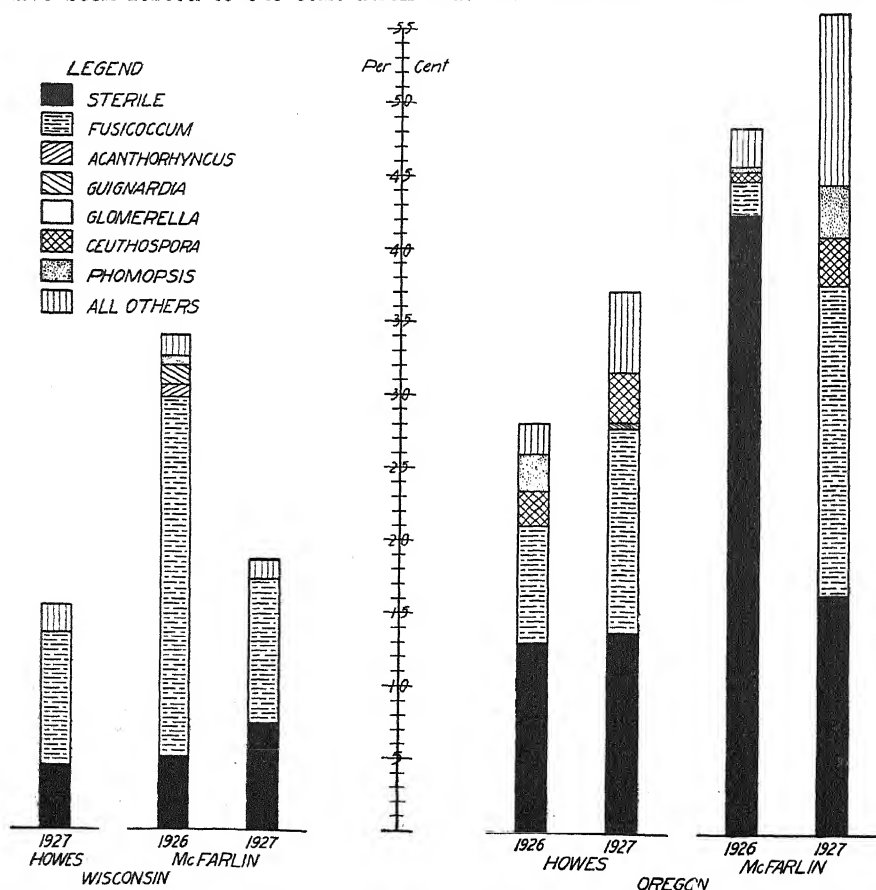


Fig. 1. Total percentage of spoilage to January 1 in the Chicago cranberry storage tests of 1926-7 and 1927-8, with proportions initiated by different fungi and sterile breakdown shown by suitable conventions.

TABLE 1.—Percentage of loss due to different fungi and other causes in the *Howes* and *McFarlin* varieties of cranberries from Massachusetts, New Jersey, Wisconsin and Oregon, in the 1926 and 1927 Chicago storage tests, to January 1 of the following year

Cause of loss	Average loss in per cent in both varieties			
	Massachusetts and New Jersey		All four states	
	1926	1927	1926	1927
Sterile	7.41	9.39	12.80	9.90
<i>Fusicoccum putrefaciens</i> Shear.....	9.99	11.35	10.80	12.50
<i>Acanthorhynchus vaccinii</i> Shear.....	1.75	1.40	1.20	0.80
<i>Guignardia vaccinii</i> Shear.....	3.11	15.66	2.00	7.80
<i>Glomerella rufomaculans vaccinii</i> Shear.....	0.77	3.42	0.40	1.70
<i>Ceuthospora lunata</i> Shear.....	—	—	0.40	0.80
<i>Phomopsis</i> sp.	0.81	0.72	1.00	0.80
Total spoiled	26.96	45.27	31.10	38.60

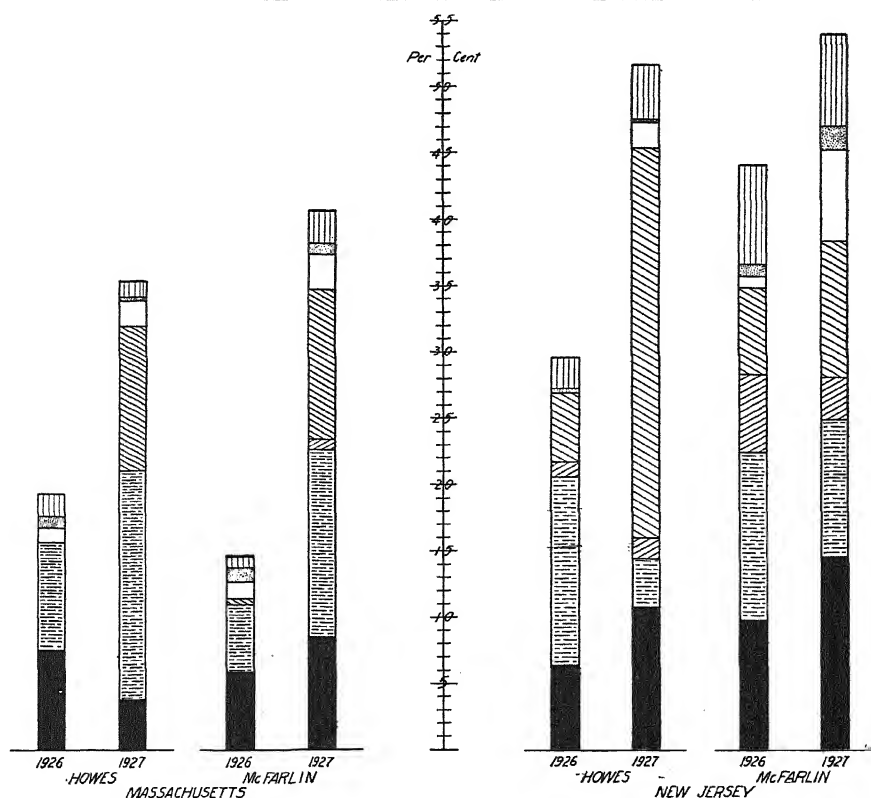


FIG. 1. (continued.) Total percentage of spoilage to January 1 in the Chicago cranberry storage tests of 1926-7 and 1927-8, with proportions initiated by different fungi and sterile breakdown shown by suitable conventions.

from Massachusetts and New Jersey berries in the two years is due to the difference in the temperature at which the berries were shipped and stored. The evidence on this point may be summarized as follows:

First—The condition of the berries as shown by holding tests in 1926 and again in 1927. A number of lots of berries of different varieties from various bogs in Plymouth County, Massachusetts, were assembled and held in basement storage at East Wareham; a somewhat smaller number of lots of New Jersey berries in a field laboratory in Toms River. The Massachusetts lots were sorted and counted on November 15 of both years. The New Jersey lots were sorted and counted November 12. An average of 14 strictly comparable lots from Massachusetts gave 12.3 per cent spoilage in 1926 and 7.9 per cent spoilage in 1927. From these results it is apparent that the condition of Massachusetts berries when harvested must have been slightly better in 1927 than in 1926. Almost identical counts were obtained in the 1926 and 1927 lots taken from the same sections as the Chicago test berries. The New Jersey berries in the holding tests also showed better keeping quality, in some cases, in 1927 than in 1926, but in the particular lot of Howes used in the storage experiment in Chicago there was slightly more rot in 1927 than in 1926.

Second—The prevailing temperature during shipping and at Chicago. As the berries were shipped and held under strictly commercial conditions, it was not possible to control the temperatures or even to record them exactly. The mean monthly temperatures for East Wareham and Boston, Mass., for Lakewood, N. J., and Chicago, Ill., show, however, that the temperature averaged from 4 to 5° F. higher during October and November of 1927 than in 1926. The approximate storage temperatures for the two years in Chicago are given in table 2.

That a difference in temperature of 4–5° F. is sufficient to account for the difference in amount of decay is indicated by the known effect of temperature on the fungi concerned and on the cranberries themselves. The careful studies of Morse and Jones³ (p. 81) show that within the temperature ranges covered by this storage test the rate of respiration as measured by carbon dioxide given off approximately doubles with a rise in temperature of 10° C. (18° F.).

Fusicoccum putrefaciens,⁴ which, when all the lots are considered, is the most destructive fungus in storage, is able to grow somewhat at 32° F. and is the only important rot fungus capable of appreciable growth below 50° F. While it grows more rapidly at temperatures above 50° F., its rate of

³ MORSE, F. W., and C. P. JONES. Studies of cranberries during storage. Mass. Agr. Exp. Sta. Bul. 198. 1920.

⁴ STEVENS, N. E. Temperatures of the cranberry regions of the United States in relation to the growth of certain fungi. Jour. Agr. Res. 11: 521–529. 1917.

TABLE 2.—*Approximate storage temperatures, Chicago, in degrees F.*

Month	1926	1927
October	65-55	70-60
November	53-43	58-48
December	40-33	48-35

acceleration is less than that of many other cranberry rot fungi. For example, its rate of growth increases less than two and one-half times from 50 to 68° F., while that of *Guignardia* increases three times and *Glomerella* six times with the same rise in temperature. During more than half the storage period of 1926 the temperature was too low for *Guignardia* and *Glomerella* to grow appreciably and these two fungi could grow but slowly earlier in the season. The cultures showed that these fungi caused a relatively small percentage of the rot in 1926. The 1927 storage temperatures were more favorable to *Guignardia* and *Glomerella*, and the cultures showed that in nearly every case the increase in these two fungi would largely account for the total increase in rot during 1927 over that in 1926.

Third—Further confirmation of the idea that the difference in storage temperatures adequately explains the different results for the two seasons is found in the fact that duplicate lots from Massachusetts stored at higher temperatures in Washington, D. C., showed a still greater amount of spoilage with corresponding increases in the percentage of the various fungi.

The writers must admit failure in their primary object, which was to secure an adequate basis for comparison of the fungi present in the cranberry crops of similar areas during successive years. The difference in shipping and storage temperatures have obscured any difference that may have existed at the time of harvest. The results of the test are of interest as showing exactly what causes of decay were active under actual commercial conditions in 1927 as compared with 1926.

SUMMARY

Cranberry storage tests with two varieties of berries from the four principal growing regions of the country were carried out in 1927 under the same commercial conditions as in 1926.

Compared with 1926, a greater amount of spoilage developed in 1927 in Massachusetts, New Jersey, and Oregon berries and a smaller amount in Wisconsin berries.

The increase in spoilage of the 1927 berries over that in 1926 was due chiefly to an increase in fungous rots, principally those that are most active at higher temperatures.

Holding tests on the bogs, on the contrary, indicated that both Massachusetts and New Jersey berries were actually sounder when picked in 1927 than in 1926.

Analysis of temperatures disclosed that the shipping and storage season for cranberries averaged from 4 to 5° F. warmer per day in 1927 than in 1926.

The increased amount of spoilage in 1927 is evidently attributable to the warmer season rather than to greater initial infection of the berries.

BUREAU OF PLANT INDUSTRY,

UNITED STATES DEPARTMENT OF AGRICULTURE,

AND

WISCONSIN DEPARTMENT OF AGRICULTURE.

NOTE ON THE OCCURRENCE OF INTRACELLULAR BODIES IN SPIKE DISEASE OF SANDAL (*SANTALUM ALBUM* LINN.)

M. J. NARASIMHAN

The spike disease of sandal has been known to occur in South India for the last 30 years, causing an annual loss of six lacs of rupees or about 150,000 dollars. The main symptoms of the disease are: reduction in the size of the leaves, shortening of the internodes, phyllody of the flowers, and death of the root ends and haustoria, leading to the death of the tree. The most striking feature is the accumulation of starch in the leaves and twigs.



FIG. 1. Photomicrograph showing the bodies (X) associated with nuclei (N) in the cells of spiked leaf of sandal. $\times 1000$.

There has been considerable literature dealing with the etiology of the spike disease of sandal, based on the assumption that unfavorable environmental conditions, such as forest fires, water-logging, drought, etc., and unsuitable host plants such as *Lantana*, played the main rôle in producing the disease, but Dr. L. C. Coleman¹ emphasized the fact that the appearance

¹ Coleman, L. C. Spike disease of sandal. Dept. Agr., Mysore, Mycol. Series, Bul. 3. 1918.

of the disease had nothing to do with the parasitic nature of sandal, and established the infectious nature of the disease by means of grafting experiments.

Cytological investigation of this disease, taken up by the author during 1927, revealed the presence of intracellular bodies in the cells of the spiked leaf. The bodies are round or oval-shaped, vacuolate, and are found in close association with the nuclei or occasionally free from them. Generally, only a single body is found in a cell, though there are cases where two to three of them occur (Figs. 1 and 2). The bodies are readily made out in



FIG. 2. Photomicrograph showing the bodies (X) free from the nuclei (N) in the spiked leaf of sandal. The vacuolated nature of the bodies can be seen. $\times 1000$.

material fixed in Flemming's weak solution, and stained with Haidenhain's iron-alum haematoxylin or Flemming's triple stain. In the latter case, the bodies take up the Orange G. Indications of the degeneration of the nuclei in some of the affected cells are not wanting. The bodies have not been found in healthy leaves so far examined.

Similar bodies (Fig. 3) have been found by the author in the leaves of *Vinca rosea* Linn. affected by a similar disease. The finding of these bodies in spiked sandal and *Vinca* indicates the similarity of the disease in the two cases, and militates against the view held by some of the workers that the spike disease in sandal is caused by its association with unsuitable host

plants. The occurrence of bodies in spiked sandal similar to those found by various workers in a number of virus diseases of plants, such as the mosaic diseases of tobacco and *Hippeastrum*, rosette disease of wheat, etc.,

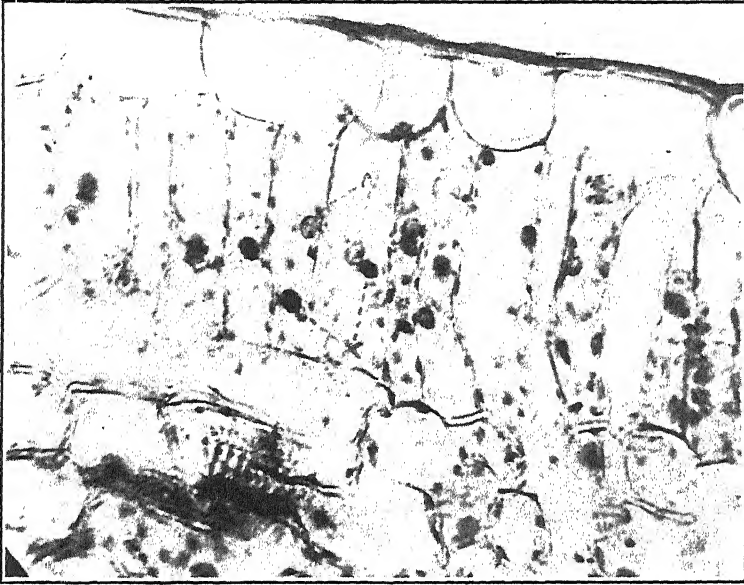


FIG. 3. Photomicrograph showing the bodies (X) in spiked leaf of *Vinca rosea*, some attached to the nucleus (N) and some free from it. $\times 500$.

and in virus diseases of animals, lends support to the virus theory of the spike disease, put forward by Dr. Coleman.

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PHYTOPATHOLOGY

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PATHOGENICITY OF *BACILLUS MESENERICUS*, *B. AROIDEAE*, *B. CAROTOVORUS*, AND *B. PHYTOPHTHORUS* TO POTATO TUBERS

PHILIP BRIERLEY

A wound rot of stored potatoes was brought to the writer's attention by R. C. Wright in March, 1924. Some tubers that had been indexed for virus diseases and left in the laboratory for a day or two to cork over before being returned to cold storage developed a soft rot at the wounds made in indexing. The rotted tissue was white to cream colored, or brown at the margin. The texture was soft but elastic, not watery. No odor was detected. Two types of bacteria transferred from dilution were tested for pathogenicity to tubers. One of these proved pathogenic, was reisolated, and the reisolated organism again produced an active rot. Miss L. C. Cash,¹ of the Bureau of Plant Industry, U. S. Department of Agriculture, has referred this isolation to *Bacillus mesentericus* (Flügge) Migula.

Preliminary tests of pathogenicity were made by inoculating pure cultures of *Bacillus mesentericus* into needle wounds in sound tubers supported over water in loosely covered crocks at room temperature (about 20° C.). A black jelly-like rot developed in the form of a funnel about the needle prick. Sometimes this dried out after little progress, but at others it destroyed the entire tuber in from a week to ten days. The decayed tissue was usually covered with a brown, wrinkled surface growth and gave off a characteristic odor suggestive of mice. A culture of the blackleg *Bacillus* tested in parallel produced comparable amounts of rot at room temperature. As opportunity permitted, the comparison was extended to include two additional species of bacteria, various temperatures, and several varieties of potatoes. The work, still incomplete in many respects, has now been discontinued.

Parasitism of spore-bearing bacteria for potato tubers has been claimed by several workers but the evidence has not been generally accepted.

¹ The writer is indebted to Miss Cash for her determination of this organism, and also to Dr. J. I. Lauritzen for the use of inoculation chambers and for the records of temperature and humidity in these chambers during the experiments herein reported.

Kramer (8) early described a spore-bearing rod as the cause of a wet rot of tubers in Germany, but his evidence for pathogenicity of the organism rests on its capacity to decompose tubers immersed in potato extract cultures held at 35° C. for from 8 to 20 days. *Bacillus mesentericus vulgatus* is one of the bacteria reported by Lepoutre (10) to attack potato tubers. Van Hall (3) found *Bacillus subtilis* and *B. vulgatus* parasitic to potato tubers and to certain vegetables and nuts at high temperatures. He made isolations from the rot that developed when samples of garden soils were incubated on slices of potato, at 23°, 30°, 37°, and 42° C. *B. subtilis*, *B. vulgatus*, or both, were always recovered from the potato slices that rotted at 37° and 42°; no infection resulted at 23° or 30° C. When potato slices were inoculated with pure cultures of these organisms, both produced marked decay at 30° and 37° and *B. subtilis* invaded weakly at 23° C. The greater virulence and wider temperature range exhibited by the pure cultures was attributed to mass action.

Smith (19) has criticized this evidence for pathogenicity of spore-bearers on the grounds that the infection tests were made under conditions abnormal for the host and that none of the investigators showed pathogenicity of a strain derived from a single well-identified spore. This objection was met at least in part by the following simple test. Three freshly made subcultures in beef broth of the strain of *Bacillus mesentericus* under study, (a) unheated, (b) heated to 80° C. for 15 minutes, and (c) heated to 100° C. for 15 minutes, were incubated at room temperature. After 48 hours subcultures (a) and (b) showed the clouding and pellicle typical of the strain; (c) remained sterile, indicating considerably less heat resistance in this pathogenic strain than has been shown for some other strains of *B. mesentericus* (11). Dilution plates were made from (a) and (b), a single well-separated colony of each was fished, and the resulting cultures used to inoculate potatoes. Three tubers of the variety Earliest of All and two of Triumph were inoculated with each of these subcultures and a like number held as controls. After seven days' incubation at 30° C. typical rot 20–40 mm. in diameter developed in each of the inoculated tubers, and the controls remained sound (Fig. 1). No change in virulence was detected in the subculture heated to 80° C., which shows that the parasitic strain of *B. mesentericus* concerned here is considerably more resistant to heat than other known bacterial-tuber-rot organisms.

A single strain of *B. mesentericus* was used in this test and all other studies reported in this paper. This strain has never been single-spored but its purity has been repeatedly confirmed by the poured-plate method. No change in virulence has been detected during four years of cultivation on artificial media. A second isolation made from Virginia Irish Cobbler

potatoes by Freeman Weiss has proved equally virulent to tubers. No survey has been attempted, and it is not known whether pathogenicity to potatoes is a characteristic of *Bacillus mesentericus* as a species or of specialized strains only. The species has been recorded as present in mixed infections of blackleg rot and of bacterial ring disease by Paine and Haenseler (16) and Spieckermann (22) respectively, but neither tested it for pathogenicity. *B. mesentericus* is so common on imperfectly sterilized potato tissue that it is sometimes called the potato bacillus in American and European texts on bacteriology, but no previous mention of its pathogenicity has been found.

The three species of bacteria compared with *Bacillus mesentericus* as a measure of its pathogenicity under various conditions were *B. phytophthorus*, *B. carotovorus*, and *B. aroideae*. The identity of the culture here referred to as *B. phytophthorus* Appel (*B. atrosepticus* van Hall) which was isolated by the writer from a blackleg infected potato plant in a greenhouse at Arlington Farm, Va., has not been fully confirmed, but it is assumed to be the blackleg bacillus from its source and behavior. A subculture of

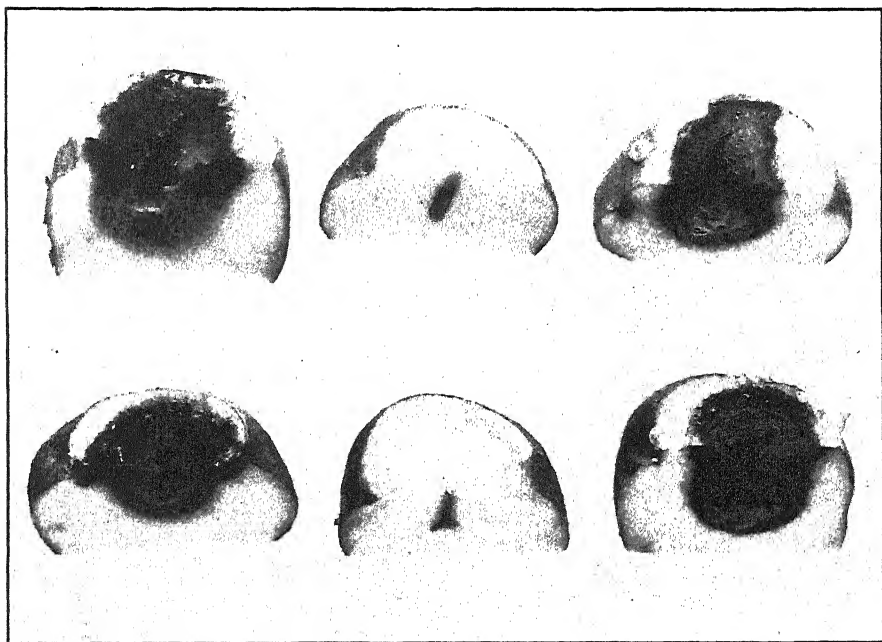


FIG. 1. Typical aspect of potato tuber rot caused by *Bacillus mesentericus*. Upper row: Triumph variety; lower row: Earliest of All. Left: Inoculated with unheated culture; center: control tubers pricked with a sterile needle (the darkening is due to wound cork formation); right: inoculated with a culture previously heated to 80° C. for 15 minutes. Incubated at 30° C.

Jones's original strain 3A of *B. carotovorus* Jones was obtained from J. I. Lauritzen, and a culture of *B. aroideae* Townsend from E. F. Smith's laboratory.

Bacillus carotovorus is now commonly named (18) as the cause of "slimy soft rot" of potato tubers, a loose term applied to bacterial soft rots, aside from blackleg, which commonly occur under conditions of handling and storage unfavorable to the tubers. Jones (6) described this organism as non-pathogenic to tubers under rigid test conditions, but other workers (9, 12, 21) have found it pathogenic in laboratory tests, and recently Lacey (9) has identified it as the cause of a soft rot of tubers under commercial conditions.

Bacillus aroideae has not been reported from soft-rotted potatoes under natural conditions, but several authors (12, 21, 23) report it pathogenic to tubers upon inoculation. This species has been reduced to synonymy with *B. carotovorus* (*Erwinea carotovora* (Jones) S. A. B.) by the Society of American Bacteriologists' Committee (1) on the basis of the findings of Harding and Morse (4). These findings show several minor differences in cultural characteristics between the two organisms which Harding and Morse considered insufficient to separate species. These authors made no tests of pathogenicity. Massey (12) has recently reopened the question by pointing out that *B. carotovorus* and *B. aroideae* differ in several respects, notably in their effect on the calla lily.²

TEMPERATURE RELATIONS ON CULTURE MEDIA

The four species, *Bacillus aroideae*, *B. carotovorus*, *B. phytophthorus*, and *B. mesentericus*, all grow well on beef agar and in beef broth. Tubes of broth or agar were inoculated with a 2-mm. loop from young broth cultures and placed at once at test temperatures. From two to five tubes of each organism were incubated at each temperature in each test, and all tests were repeated one or more times. The cardinal points for growth, which were alike for the solid and liquid media, are shown in table 1. At the lower temperatures negative records indicate no growth in three months. Temperatures given are the average of recorded observations to the nearest half degree Centigrade. The fluctuation was never more than 1° C. in any tests used in the compilation of the table.

Previous workers with *Bacillus phytophthorus* (13, 17, 20) agree that the minimum temperature for this organism is below 5° C. Smith (20) reports little growth below 4-5° C. The writer found a trace of growth on agar and distinct clouding of broth in three months at 2° C. The

² Link and Taliaferro (Bot. Gaz. 85: 198. 1928) report that "Serologically *B. aroideae* and *B. carotovorus* are distinct, although closely related."

TABLE 1.—*Temperature relations of tuber-rot bacteria in beef bouillon and on beef agar*

Organism	Minimum °C.		Optimum °C.	Maximum °C.	
	Negative	Positive		Positive	Negative
<i>B. phytophthorus</i>	0	2	28-32	34.5	37
<i>B. carotovorus</i>	0	2	28-34	37	39
<i>B. aroideae</i>	2	5.5	28-37	39	42
<i>B. mesentericus</i>	5.5	8	39-42	49	52.5

optimum as measured by rate of clouding broth was 28-32° in these tests; 25° or 28-30° according to others (1, 13, 17, 20). The maximum reported by Morse (13) is between 32° and 33°, by Shapovalov and Edson (17) between 33° and 35°, by Smith (20) 36° C. For *B. carotovorus* Jones (6) gives maximum 38°, optimum 27-30°, and minimum near 4°, negative at 1° C. For *B. aroideae* Townsend (23) gives minimum 6°, optimum 35°, maximum 41° C. The writer's records are in general accord with published reports in all cases. The data are presented here in tabular form to show the ascending sequence of cardinal temperatures from *B. phytophthorus* to *B. mesentericus*, a sequence that also holds for the growth of these organisms on potato tubers.

EFFECT OF TEMPERATURE ON DEVELOPMENT OF TUBER ROTS

Temperature relations of the bacteria on raw potato were determined by inoculating whole tubers and incubating them in special chambers in which temperature and humidity were under control.³ A lot of five tubers in a separate 4-quart till basket was used for each organism and for a control at each temperature. A slice was cut from one end of each tuber with a sterile knife and this freshly cut surface was pricked with a heavy platinum needle bearing surface growth from a young agar culture. Control tubers were pricked in like manner with a sterile needle. Temperatures are presented as the average to the nearest half degree Centigrade and relative humidities as the average to the nearest per cent. Fluctuation during an experiment rarely exceeded 1° C. in temperature or 5 per cent in relative humidity.

A preliminary test of the four pathogens on the Green Mountain variety through a range of 8° to 34° C. showed *Bacillus aroideae* and *B. mesentericus* to be virulent at temperatures above 23° C., *B. phytophthorus* active at 8°-34° but especially at 8°-25°, and *B. carotovorus* weakly parasitic throughout.

³ Wooden chambers about one meter in each dimension, with electrical heat control, air circulation, and manual regulation of humidity, with a permanent wet and dry bulb device for reading humidity in place, were available. Records of temperature and humidity were made twice daily.

This experiment was repeated with tubers of the White Rural variety. A slice was cut from the bud end and inoculation made by pricking a 3-day agar culture into the tuber to a depth of 3–3.5 cm. at right angles to the cut surfaces. The incubation period was 10 days. As a criterion of amount of rot two diameters were measured, one at the surface, the other parallel to the surface at a depth of 1 cm. Both measures are desirable in presenting an index of the amount of actual rot because of the different character of invasion by the several organisms. *Bacillus carotovorus*, *B. mesentericus*, and under some conditions *B. aroideae* may form craters or funnel-shaped cavities which decrease in diameter below the surface. On the other hand, the rot caused by *B. phytophthorus* frequently increases in diameter as the depth increases.

Plates XIX and XX show the character of rot resulting in this test after three days at eight different temperatures. Lateral extension of the cavity below the surface is shown in *B. phytophthorus* rot at 25° C. *B. aroideae* and *B. carotovorus* formed craters or funnels of rot at the lower temperatures, and *B. mesentericus* produced this type of cavity throughout the range. The photographs fail to differentiate clearly between cork formation or dark stains and actual rot. *B. phytophthorus* and *B. carotovorus* produced definite rot at all temperatures, *B. aroideae* a bare trace at 8° and definite invasion at higher temperatures, but *B. mesentericus* is definitely negative at 8° and made only a trace of development at 12° and 15.5°, the first definite rot appearing at 16.5° C.

Of the five tubers inoculated with each organism at each temperature, one was removed for the illustrations in Plates XIX and XX; the other four were cut and measured after 10 days. These measurements are shown in table 2. Where the rot was still active at the end of 10 days the figures are shown in bold face. The turgid cortex about a rotted pith which has been mentioned by Shapovalov and Edson (17) as characteristic of blackleg tuber rot and by Lacey (9) of *B. carotovorus* was noticeable in this experiment at 29° in the tubers inoculated with *B. aroideae*, *B. carotovorus*, and *B. phytophthorus*, but was absent in the lot inoculated with *B. mesentericus*.

The effect of temperature on the development of these rots was tested further in March, 1926. The same methods were used as in the previous experiment but the range of temperature was extended to 2° C., and two varieties, Green Mountain and White Rural, were included. The test lots were all distributed to the proper incubation chambers the day before inoculation to allow them to reach the desired temperature. They were then removed one lot at a time for inoculation and replaced at once. The test was concluded after seven days at the temperatures 15° to 33°, but was left for

TABLE 2.—*Effect of temperature on development of bacterial tuber rots in White Rural potatoes. [Bold face figures indicate that the rot was still active at the end of test.]*

Temperature in degrees C.	Relative humidity, per cent.	Average diameter of rot in mm. in 4 tuber samples after 10 days										Check 5 tubers
		<i>B. mesen- tericus</i>		<i>B. caroto- vorus</i>		<i>B. phyto- phthorus</i>		<i>B. aroideae</i>				
		Sur- face	1 cm. deep	Sur- face	1 cm. deep	Sur- face	1 cm. deep	Sur- face	1 cm. deep			
8	90	0	0	11	0	24	13	7	0	Sound		
12	94	7	0	13	6	20	21	8	6	do		
15.5	93	11	0	10	5	23	27	11	22	do		
16.5	93	13	3	11	8	26	25	14	18	do		
21	90	13	20	13	15	19	25	16	24	do		
25	91	21	30	14	18	24	31	20	31	do		
29	73	38	41	16	30	15	29	29	38	do		
33.5	91	40	41	9	9	3	10	40	47	do		

42 days at the lower range of 2° to 11° C. The results for both varieties are shown in table 3.

Marked differences in the effect of temperature on progress of the four types of rot appear from the data in tables 2 and 3. *Bacillus phytophthorus* invaded tubers at 2°–33.5° C. and proved destructive at 5.5°–29° C.; *B. carotovorus* attacked potatoes at 8°–33.5°, but was of little importance outside a narrow range between about 21° and 29°; *B. aroideae* was active from 11° up, and particularly virulent from 20° up; and *B. mesentericus* was even more distinctly a high temperature form, invading but feebly at 12°–16.5°, but a vigorous pathogen above 19° C. Both the high temperature forms were able to advance unchecked at favorable temperatures.

Few tests of the behavior of tuber rot bacteria at different temperatures have been published. Paine (14) reported that blackleg rot was often corked out of potato slices at 12°–14° C., but decomposed the whole slice at 20° C. Paine and Chaudhuri (15) in a study of *B. solanisaprus* and *B. atrosepticus*, both blackleg organisms and regarded as synonymous by some authors (5, 13), found the latter failed to infect at 30° but the former produced slight rot. Their table shows a steadily increasing amount of rot produced on slices at 12°–33° C. The results presented here show a less important effect of temperature on the activity of the blackleg organism, possibly due to different relations of cork formation in whole tubers as compared to slices. No previous study of the temperature relations of the other species of bacteria on potato has been seen by the writer.

The apparent decline in activity of *B. carotovorus* and particularly *B. phytophthorus* at the higher temperatures and the increasing activity

TABLE 3.—*Effect of temperature on development of bacterial tuber rots in White Rural and Green Mountain potatoes. Incubation period 7 days at 15° and above, 42 days at 11° and below. [Bold face figures indicate that the rot was still active at the end of the test.]*

Temperature in degrees C.	Relative humidity per cent.	Average diam. of rot in mm. in 5 tuber samples									
		White Rural									
		<i>B. mesentericus</i>		<i>B. carotovorus</i>		<i>B. phytophthorus</i>		<i>B. aroidae</i>		Check	
		Surface	1 cm.	Surface	1 cm.	Surface	1 cm.	Surface	1 cm.		
2	99	0	0	0	0	0	0	0	0	Sound	
5.5	98	0	0	0	0	8	5	0	0	do	
8	93	0	0	0	0	8	8	0	0	do	
11	97	0	0	0	0	11	6	11	4	do	
15.5	94	7	0	6	0	8	6	18	13	do	
19	95	16	5	5	4	9	9	17	16	do	
22.5	90	24	23	11	10	10	4	30	28	do	
28.5	92	46	39	21	24	0	0	48	47	do	
33	95	56	47	Contam.		Contam.		63	63	Contam.	

		Green Mountain									
2	99	0	0	0	0	9	5	0	0	Sound	
5.5	98	0	0	0	0	11	5	0	0	do	
8	93	0	0	0	0	12	6	0	0	do	
11	97	0	0	0	0	5	5	3	7	do	
15.5	94	7	0	8	0	9	5	11	6	do	
19	95	12	trace	9	0	7	4	10	8	do	
22.5	90	25	12	0	0	6	5	16	14	do	
28.5	92	34	29	6	7	4	4	39	42	do	
33	95	49	32	13	4	0	0	60	49	3 sound, 2 contam.	
		(3 contam.)		(1 contam.)		(2 contam.)		(3 contam.)			

of the other two species suggested tests at a still higher range. Four half tubers inoculated with a given culture and four corresponding halves pricked with a sterile needle were placed in covered glass dishes at six temperatures. At 25° and 30.5° typical rot was produced by all four organisms while the check tubers remained sound. At 37° the checks remained sound and the high temperature forms, *B. aroideae* and *B. mesentericus*, developed normal rot. *B. carotovorus* showed a trace of rot. *B. phytophthorus* proved clearly negative in one test at this temperature, but in a second trial the tissues about the inoculation point were blackened as in incipient rot. At 39° C. tubers inoculated with *B. phytophthorus* and

B. carotovorus remained sound except for slight local staining; the checks also were sound. *B. aroideae* and *B. mesentericus* invaded rapidly at 39°, with typical symptoms, but uninoculated checks frequently became contaminated. Both these organisms were reisolated from inoculated half tubers held at 39° and *B. mesentericus* was isolated from an uninoculated check half tuber incubated adjacent to potatoes inoculated with *B. mesentericus*. At 42° *B. mesentericus* rot was still typical in appearance and odor but the status of *B. aroideae* has not been clearly determined. Rot followed inoculation with this organism at 42° but uninoculated tubers also decomposed with a mixed population of organisms present. Tubers held in covered glass dishes at 49° C. showed heat necrosis throughout in 48 hours so that no index of parasitism was available. It appears that *B. mesentericus* rots potatoes vigorously at 20° C. and up to temperatures that are fatal to the tubers; *B. aroideae* is an active parasite as high as 39° C., possibly higher; *B. carotovorus* and *B. phytophthorus* are of minor importance, if any, at temperatures of 37° C. and above.

The four bacterial pathogenes considered fall into two fairly definite groups. *B. aroideae* and *B. mesentericus* are rapidly destructive from 20° C. to temperatures in excess of 35° C. *B. carotovorus* and *B. phytophthorus* have lower temperature requirements, which make them, especially the latter, dangerous to potatoes in cool storage. The two high temperature forms are of great potential importance to tubers which become heated in transit or in poor storage. *B. aroideae* in particular may be found destructive under field conditions also, when a survey of soft rots in the field is undertaken.

EFFECT OF HUMIDITY ON BACTERIAL TUBER ROTS

In the preliminary pathogenicity tests tubers were supported over water in 1-gallon crocks with loose-fitting covers and incubated at room temperature. Inoculation was made by needle prick, introducing young agar cultures through a freshly cut surface. This method afforded satisfactory results in the form of typical rot and sound checks. When similar inoculum was smeared on a cut surface rather than pricked into it, uniformly successful infection resulted with the virulent organisms, but the extent of rot was slightly less. When either smear or needle prick inoculations were made and the potatoes incubated at room temperature in crocks without water, infection was distinctly restricted. *B. aroideae*, *B. carotovorus*, and *B. phytophthorus* made no advance from smears, and their slight progress from the needle pricks was corked out. *B. mesentericus* made slight progress from the smears, which was stopped in all cases; in the needle pricks invasion took place in all cases but was blocked in four out of six.

TABLE 4.—Effect of humidity at 12° and 22° C. on progress of tuber rots caused by 4 species of bacteria. Number of tubers showing infection and average diameter of rot in mm. at surface and at 1 cm. depth in 5 tuber samples. Irish Cobbler, inoculated April 21, measured May 10, 1927. [Bold face figures indicate that the rot was still active at close of experiment.]

Inoculum	Tuber area observed	12° C.						22° C.					
		Relative humidity in per cent											
		87	74	67	55	94	90	82	78				
		No. Diam.	No. Diam.	No. Diam.	No. Diam.	No. Diam.	No. Diam.	No. Diam.	No. Diam.	No. Diam.	No. Diam.	No. Diam.	No. Diam.
<i>B. mesentericus</i>	Surface 1 cm. deep	5 7 0 ..	0 .. 0 ..	1 6 0 ..	0 .. 0 ..	5 14 5 5	5 10 5 4	5 11 5 7	5 8 5 3				
<i>B. carotovorus</i>	Surface 1 cm. deep	5 7 0 ..	0 .. 0 ..	0 .. 0 ..	0 .. 0 ..	2 9 1 2	2 6 0 ..	2 5 1 2	0 .. 0 ..				
<i>B. phytophthorus</i>	Surface 1 cm. deep	5 21 5 12	5 20 5 10	5 16 5 9	5 16 5 11	5 21 5 9	5 18 5 10	5 12 5 6	5 8 5 6				
<i>B. aroideae</i>	Surface 1 cm. deep	1 12 1 5	5 6 2 6	1 6 1 4	1 6 0 ..	5 11 5 3	5 7 5 3	3 5 3 3	5 8 4 3				

To study more precisely the effect of humidity on the progress of these rots, the control chambers mentioned previously were again utilized. A series of four controlled humidities at 12° and a similar series at 22° C. were available. Each of the four bacterial pathogens was inoculated into five Irish Cobbler tubers by needle pricks through a freshly cut surface, and these were placed together with five similar tubers pricked with a sterile needle in each of the eight control chambers. Fluctuations in control during this experiment were less than 2° C. in temperature and 5 per cent in humidity except that the two low humidities at 12° varied slightly more. The results of the test are summarized in table 4.

B. carotovorus produced negligible infection throughout and in all cases was stopped at the end of the 19-day period. *B. aroideae* produced little infection at 12°. At 22° this organism initiated rot in a majority of inoculated tubers, but was no longer active in any at the end of the test. *B. mesentericus* failed almost completely at 12°, but entered in all cases at 22° and remained active at 80 per cent humidity. *B. phytophthorus* was the most consistently successful invader throughout the test, and proved vigorous at the lowest humidity employed at 12°, but was inhibited by low humidity at 22° C.

Considering the method of inoculation employed in this test, low humidity could not be expected to prevent infection by a virulent pathogene inasmuch as the bacteria were forced deep into the moist tissues of the potato and could not become subject to drying for some time after inoculation. Neglecting the weak pathogene *B. carotovorus* and the performance of *B. mesentericus* at 12°, which is below the range of activity for this species, no effect of humidity on number of infections was evident. Diameter of penetration, on the other hand, is a measure of the ability of the parasite to continue activity under the test conditions. Here a general tendency appeared toward diminished penetration with lower humidity, a trend which applied to all four organisms. The few cases in which rot remained active at the end of the test were at humidities above 80 per cent. The general indication is that low humidities restricted advance of these rots, but failed to check completely the advance of a virulent pathogene in a deep wound.

REACTION OF POTATO VARIETIES TO BACTERIAL TUBER ROTS

No previous study of the reaction of potato varieties to soft rots other than blackleg rot has been seen by the writer. Work on varietal susceptibility to blackleg has been reviewed by Kotila and Coons (7). These authors conclude from their studies that no variety is resistant enough to preclude loss, and that tuber reaction is not correlated with stem susceptibility. On

the basis of tuber susceptibility they list the following varieties in order, beginning with most resistant: Triumph, Green Mountain, Russet Rural, Irish Cobbler, Early Ohio, Sir Walter Raleigh, Carman No. 3.

In the present work ten varieties were tested at least once, some three times, for susceptibility to four bacterial rots. For the first and second tests run in January, 1926, the lots of Cobbler, Russet Rural, White Rural, and mature Green Mountain were grown in one field in Pennsylvania and were strictly comparable; Spaulding Rose came from another section of Pennsylvania, Triumph from Maine, McCormick and immature Green Mountain from Arlington Farm, Va. The third test, run a year later, included Cobbler, Russet Rural, Green Mountain, Spaulding Rose, Triumph, and Early Rose from one field in Pennsylvania, Early Ohio and Burbank from Maine, and McCormick from Arlington Farm. A stock of Green Mountain grown in Vermont in 1925 and stored about 18 months at 4.5° C. was added. Inoculation in the first and third tests was made by pricking a young agar culture into the tubers through a freshly cut surface to a depth of about 3 cm. by means of a heavy platinum needle. In the second test 1 cc. of a 3-day broth culture was poured into a well 8 mm. in diameter and from 2 to 3 cm. deep in each tuber. The wells were not sealed in any way. The use of wells instead of needle wounds tended to magnify the diameter of the rot in this second test. The inoculated tubers in the first and second tests were supported over water in loosely covered 1-gallon crocks and incubated 16 days at room temperature; in the third experiment they were incubated 11 days in 4-quart baskets at 21° C. and approximately 80 per cent humidity. Because of these differences in method of inoculation and incubation the three tests were not exactly comparable, but all varieties in any one test were treated alike. Check lots of 5 tubers of each variety were included in each test; of 130 such tubers all but one remained sound.

In table 5 the varieties are listed in order of decreasing susceptibility according to their average performance with respect to all four organisms in one or more of the three experiments. These data show that the varieties Burbank, Early Ohio, Spaulding Rose, and the lot of Green Mountain from prolonged storage were more susceptible to blackleg rot than to the other three types of decay. With these exceptions the reactions of the several varieties to the four types of rot are in general of the same order. Russet Rural and White Rural were clearly most susceptible. Russet Rural alone afforded all four bacteria suitable conditions for continued rot at the comparatively low humidity used in the third test, and Russet Rural and White Rural showed less ability to block out the rots than the other varieties tested. *B. mesentericus*, *B. aroideae*, and *B. phytophthorus* were all active at the conclusion of each test with the Rural types, and *B. carotovorus* remained

TABLE 5.—*Reaction of potato varieties to bacterial tuber rots. [Average diameter of rot in mm. in 5 tuber samples. Duration of first and second tests 16 days, third test 11 days. Bold face figures indicate that the rot was still active at close of experiments.]*

Variety	<i>B. mesentericus</i>			<i>B. carotovorus</i>			<i>B. phytophthorus</i>			<i>B. aroidae</i>			Average all organisms
	1st test	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	
White Rural	27	25	10	14	29	17	32	45	25
Russet Rural	15	31	26	9	14	13	20	17	39	33	50	17	24
Burbank	8	6	41	14	17
Irish Cobbler	7	24	8	14	0	2	30	28	11	22	32	6	15
Green Mountain, 18 months old	9	7	36	22	19
Green Mountain, immature	19	18	15	0	5	10	13	35	14
Green Mountain, mature	4	16	10	8	0	5	7	26	10	9	35	9	12
Triumph	6	11	10	4	0	6	16	33	9	22	31	9	13
Early Ohio	5	5	22	8	10
Early Rose	8	5	12	8	8
Spaulding Rose	2	0	7	0	0	5	3	30	10	4	0	9	6
McGonnick	0	14	7	0	0	4	0	13	3	8	11	9	6

active once. The Irish Cobbler was relatively susceptible to all, but only blackleg gained a firm foothold in this variety in the last and least favorable test. McCormick was most resistant; Spaulding Rose consistently ward off all but blackleg rot. Green Mountain and Triumph fell in the middle ground, less susceptible than the Rurals and Cobbler but much more so than Spaulding Rose and McCormick. Burbank, Early Ohio, and Early Rose could be placed only tentatively from a single test. Little difference appeared between stocks of Green Mountain harvested when immature (Arlington) and when mature (Pennsylvania), but the lot stored for 18 months showed increased susceptibility to all the organisms. Plates XXI and XXII show the appearance of two highly susceptible and two slightly susceptible varieties at the conclusion of the first experiment. In White Rural and Cobbler all infections have made good progress, but the *B. carotovorus* is no longer active in the Rural. In Spaulding Rose *B. carotovorus* has not progressed, *B. mesentericus* and *B. aroideae* have been stopped after slight progress, and *B. phytophthorus* is still alive. In McCormick, *B. aroideae* has made distinct advance and *B. mesentericus* a trace, but both are blocked out.

PATHOGENICITY OF TUBER ROT BACTERIA FOR OTHER HOSTS

Various plants were inoculated with each of the four organisms under study, particularly to determine whether *B. mesentericus* was capable of attacking plant parts other than potato tubers. The three soft-rot organisms were included to provide a measure for the effectiveness of the conditions of inoculation and incubation. From 2 to 15 plants were inoculated with each organism and a like number of uninoculated plants were held under comparable conditions in each test. Inoculations were made by pricking young agar cultures into the plants with a sterile needle. In laboratory tests the inoculated plant parts, except the cabbage heads, were supported over water in covered crocks and incubated at room temperature. The cabbage heads were treated similarly, but no water was put in the crocks. In the greenhouse experiments the plants were incubated for from 2 to 3 days in a glass-walled moist compartment, or were covered with bell jars for a like interval, then returned to the open bench. The greenhouse temperatures usually ranged from 15 to 20° C. at night and were often higher during the day. The results of all tests are summarized in table 6. It will be seen that *B. mesentericus* proved uniformly negative on plants other than the potato, and on parts of the potato plant other than the tubers. Two doubtful cases and other observations of interest will be discussed further.

Laboratory Experiments: Carrot roots. *Bacillus carotovorus*, *B. aroideae*, and *B. phytophthorus* all caused marked decay of carrots in four

days. No consistent difference in symptoms was observed, but *B. phytophthorus* rot was in some cases darker in color. *B. aroideae* rot progressed most rapidly. Needle inoculations with *B. mesentericus* were without effect, but when a young broth culture of this organism was poured into cork borer wells in carrots, one root was invaded for 2-5 mm., two others remaining sound.

Turnip roots. The three soft-rot organisms, *B. carotovorus*, *B. aroideae*, and *B. phytophthorus* caused an active brown soft rot of turnips in four days.

Cabbage heads. Cabbage heads were rotted by the soft-rot bacteria to a diameter of 10-15 mm. along the whole depth of the needle pricks in four days. At the end of this period *B. carotovorus* and *B. phytophthorus* were still active, but the *B. aroideae* rot had dried out.

Iris versicolor. Plants of the variety Queen of May were brought into the greenhouse in March, and inoculated into the young rootstocks and at the base of the young leaves, then incubated in crocks in the laboratory. Both rootstocks and leaves were attacked by all three of the soft-rot bacteria. In five days *B. carotovorus* rot in the rootstock had dried out, *B. phytophthorus* had half destroyed the rootstock, and *B. aroideae* completely destroyed it. In young leaf bases all three caused rapid translucent watery decay, which spread upward into the leaves 2-9 cm. and brought about complete collapse of the plants.

Greenhouse Experiments: Potato stems. In the spring of 1925 cultures of the blackleg organism and of *B. carotovorus* and *B. mesentericus* were inoculated into young stems of Russet Rural potatoes. At the end of seven days the blackleg organism had killed three plants completely and a fourth was killed above the needle wound but was sprouting below. *B. carotovorus* killed one stalk but two others healed without appreciable injury. Of the two inoculations with *B. mesentericus*, one brought complete collapse, the other killed the shoot above the lesion but growth was resumed below. Two check plants pricked with a sterile needle remained sound.

This experiment was repeated twice in 1926, with *B. aroideae* added. At the end of three days' incubation under bell jars, *B. phytophthorus*, *B. aroideae*, and *B. carotovorus* had completely killed three Russet plants inoculated with each, but three plants inoculated with *B. mesentericus* and three check plants remained entirely healthy. Irish Cobbler stems reacted similarly. Two plants inoculated with *B. phytophthorus* and three with *B. aroideae* were killed in six days; *B. carotovorus* killed one, and half girdled a second, but three shoots pricked with *B. mesentericus* and one check plant remained sound. The three species producing active stem rot were indistinguishable in behavior on potato stems. The discoloration

produced by the blackleg organism was occasionally but not consistently darker than that caused by *B. aroideae* and *B. carotovorus*, but all three blackened the tissues.

Tomato stems. In six days *B. aroideae* and *B. phytophthorus* each killed two of three tomato plants inoculated, and produced local lesions which dried out in the third. *B. carotovorus* formed small local lesions which dried out without appreciable injury.

Bean stems. Nine bush bean plants were inoculated with each of four organisms. At the end of 10 days four plants inoculated with *B. carotovorus*, three with *B. phytophthorus*, and three with *B. aroideae* had softened at the needle prick and collapsed. Some of the remaining plants inoculated with each of these parasites showed local browning and cracking at the wound, but the *B. mesentericus* and check sets remained healthy.

Cucumber seedling stems. Fifteen cucumber seedlings in the two-leaf stage were inoculated about 2 cm. above the soil level with each of the four organisms, and fifteen were held as checks. In six days *B. aroideae* had softened the stems of nine plants and brought about complete collapse at the point of inoculation. No evidence of invasion by *B. carotovorus*, *B. phytophthorus* or *B. mesentericus* was detected. One check plant damped off at the soil line.

Cabbage seedling stems. Cabbage seedlings about 10 cm. tall had completely collapsed upon removal from the moist chamber two days after inoculation with *B. aroideae*, *B. carotovorus*, and *B. phytophthorus*. A translucent, watery decay extended up the stem into the leaf petioles and veins in all cases, and well into the leaf lamina in the case of *B. carotovorus* and *B. aroideae*. The plants inoculated with *B. mesentericus* and the check plants remained healthy.

Pelargonium zonale stem cuttings. Two well-rooted cuttings with five or six leaves were inoculated with each culture. In five days *B. carotovorus* killed one but did not injure the other; *B. phytophthorus* killed one and produced a local lesion on the second; *B. aroideae* killed one completely and killed the bud of the other. Plants inoculated with *B. mesentericus* remained sound; one of two check plants was killed by Botrytis infection present before incubation.

Iris versicolor leaves. Iris plants brought from an outdoor planting to the greenhouse in March were inoculated in June. When the plants were removed from the moist chamber after three days, the three soft-rot bacteria, *B. aroideae*, *B. phytophthorus*, and *B. carotovorus*, had produced marked decay, a watery zone extending upward into the leaves from the needle wound for 2-25 cm. One of the checks rotted; two others and the plants inoculated with *B. mesentericus* remained healthy. These results

are not in accord with Massey's (12) report that iris is a differential host for *B. aroideae* and *B. carotovorus*, the latter but not the former attacking it.

TABLE 6.—*Summary of inoculation tests with four bacterial pathogens on various hosts*

Host	Part inoculated	<i>Bacillus mesentericus</i>	<i>B. carotovorus</i>	<i>B. phytophthorus</i>	<i>B. aroideae</i>	Checks
<i>Laboratory tests</i>						
Carrot	Root	0 ?	+	+	+	Sound
Turnip	do	0	+	+	+	do
Cabbage	Head	0	+	+	+	do
<i>Iris versicolor</i>	Root stock and leaf bases	0	+	+	+	do
<i>Greenhouse tests</i>						
Potato	Stems	0 ?	+	+	+	do
Tomato	do	0	Trace ?	+	+	do
Bean	do	0	+	+	+	do
Cucumber	Seedling stem	0	0 ?	0 ?	+	14 sound 1 damped off
Cabbage	do	0	+	+	+	Sound
<i>Pelargonium zonale</i>	Stem cutting	0	+	+	+	1 sound 1 Botrytis
<i>Iris versicolor</i>	Base of leaf	0	+	+	+	2 sound 1 rotted
<i>Zantedeschia aethiopica</i>	Petioles	0	0	0	+	Sound

Zantedeschia aethiopica petioles. Petioles of the white calla lily were inoculated repeatedly with the four organisms under study but only *B. aroideae* produced any effect on this host. When petioles were inoculated with this organism, slight brown watery lesions appeared at the needle wound in three days and gradually increased in size. In seven to nine days blotches appeared on the leaf blades and the leaves turned yellow and collapsed. In some instances the rot spread to other leaves of the same plant and these collapsed in turn. Inoculation of this host, as already pointed out by Massey (12), is apparently a reliable means of distinguishing *B. aroideae* from *B. carotovorus*. This point should be tested further with additional strains, especially inasmuch as Bewley (2) has reported

B. carotovorus and not *B. aroideae* as the cause of soft rot of the calla lily in England.

SUMMARY

Bacillus mesentericus was isolated from a wound rot of potato tubers. Pure cultures of this organism readily produced rot in healthy potatoes at 20° C. and above. The pathogenicity of cultures was retained after heating to 80° C. for 15 minutes.

Bacillus mesentericus was found to grow on artificial media within the range 8–49° C. with an optimum at 39–42° C. Three other species pathogenic to tubers, *B. aroideae*, *B. carotovorus*, and *B. phytophthorus*, showed lower minima, optima, and maxima.

Bacillus mesentericus invaded potato tubers feebly at temperatures below 20° C., but advanced more rapidly as the temperature was increased, and showed no maximum within the range at which potatoes could be held without injury. *B. aroideae* showed a similar temperature curve, with a slightly lower minimum, and greater virulence. These two may be grouped as potentially dangerous to potatoes improperly stored, or heated in transit. *B. carotovorus* and *B. phytophthorus*, particularly the latter, showed lower minima for growth on raw potato, and also a tendency to reach a maximum near 30° C., above which they declined in activity.

At the temperatures 12° and 22° C. relative humidity had no effect on the number of infections resulting from needle inoculations into the interior of tubers, but relative humidity lower than 80 per cent checked the progress of all four bacteria at these temperatures.

The varieties Rural New Yorker and Russet Rural, followed by Irish Cobbler, were most susceptible to all four types of rot. McCormick was most resistant, and Spaulding Rose was resistant to all but blackleg rot. Five other varieties are classed between these extremes on the basis of limited data.

Bacillus mesentericus did not attack other plants or other parts of the potato than the tuber.

OFFICE OF VEGETABLE AND FORAGE DISEASES,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.

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EXPLANATION OF PLATES

PLATE XIX.

Progress of bacterial rots in White Rural potatoes at eight temperatures. Photographed three days after inoculation. Left: *Bacillus mesentericus*; right *B. aroideae*. The actual average temperatures during incubation were, reading from top to bottom, left column: 8°, 12°, 15.5°, 16.5° C.; right column: 21°, 25°, 29°, 33.5° C.

PLATE XX.

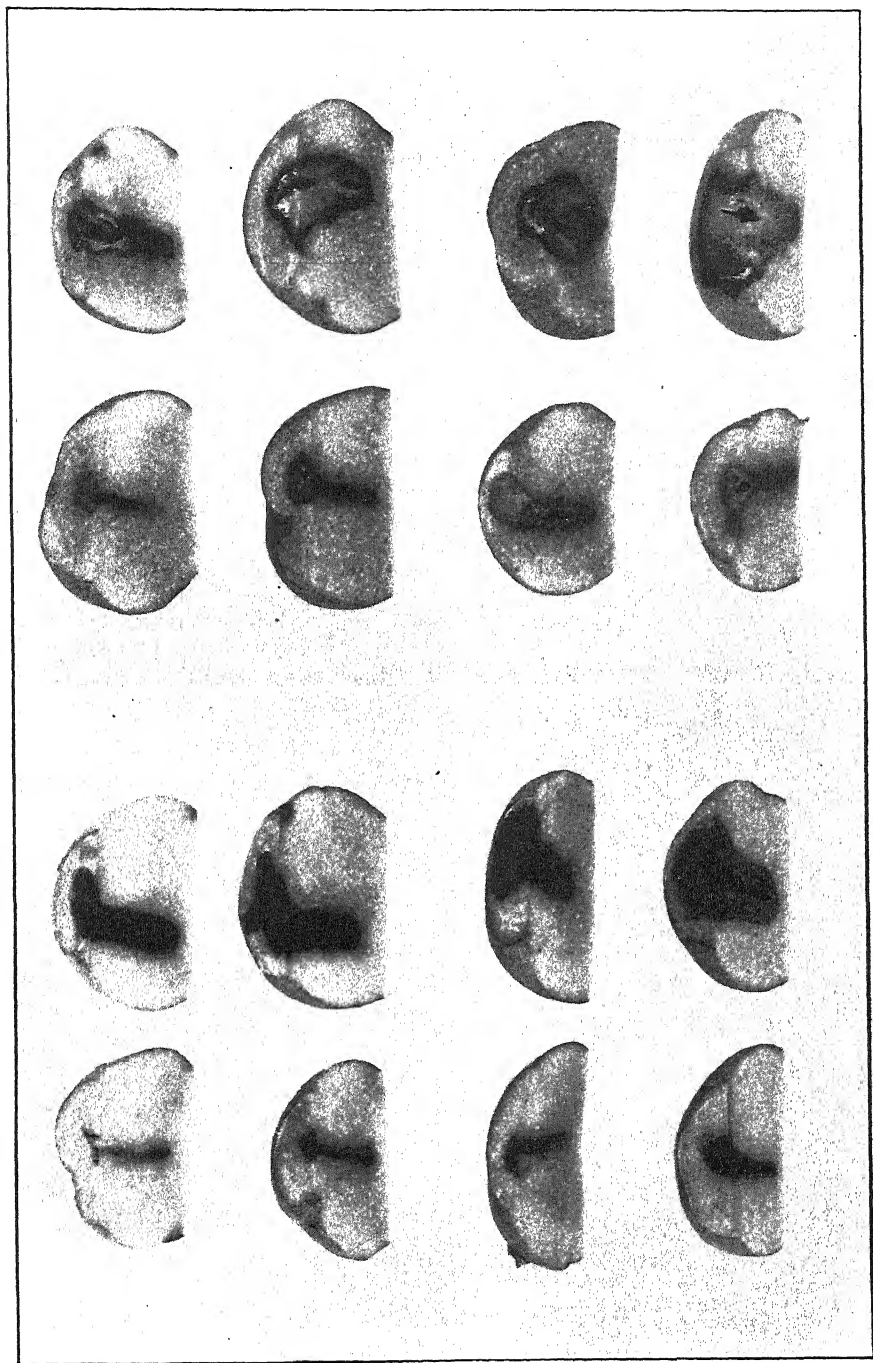
Progress of bacterial rots in White Rural potatoes at eight temperatures. Photographed three days after inoculation. Left: *Bacillus carotovorus*; right: *B. phytophthorus*. The actual average temperatures during incubation were, reading from top to bottom, left column: 8°, 12°, 15.5°, 16.5° C.; right column: 21°, 25°, 29°, 33.5° C.

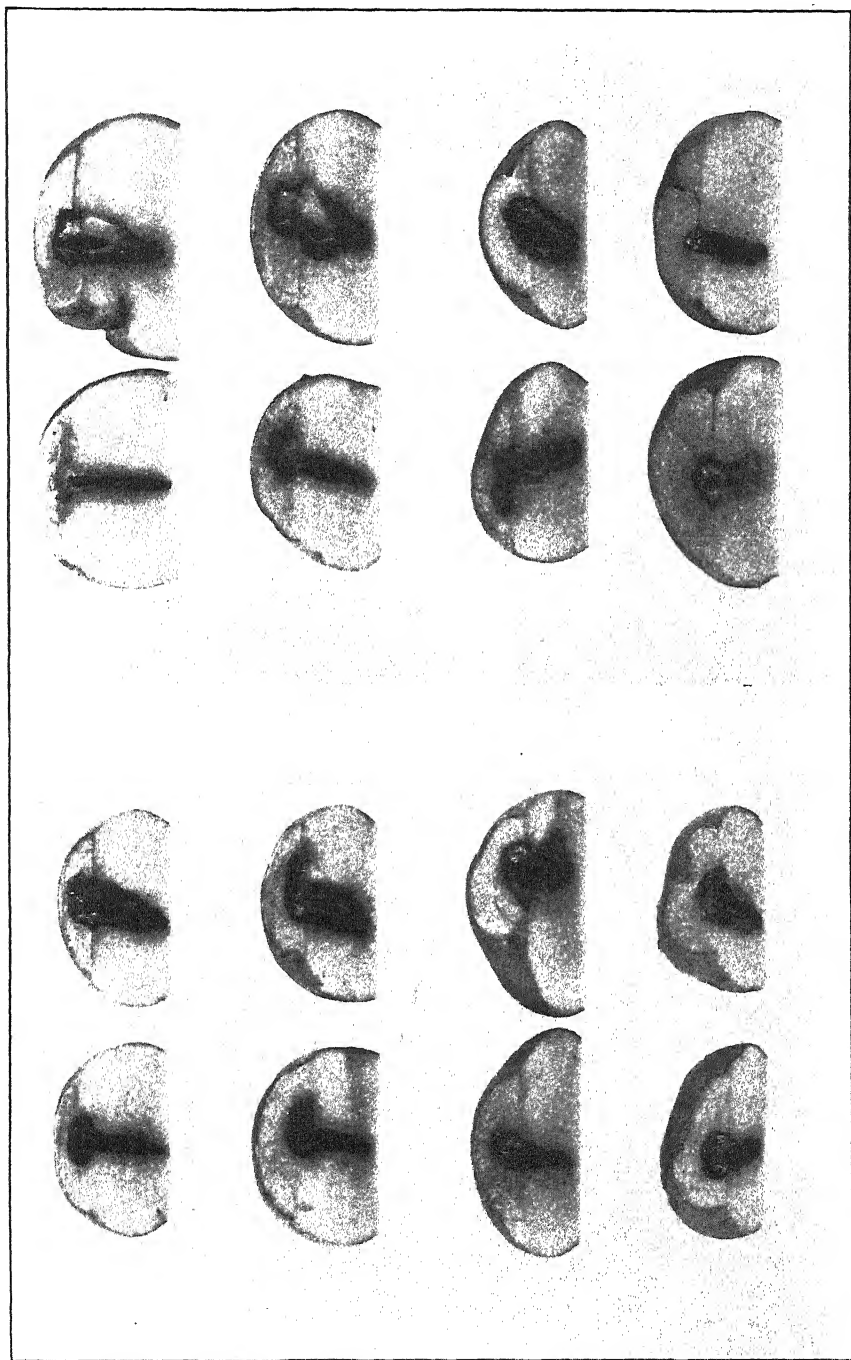
PLATE XXI.

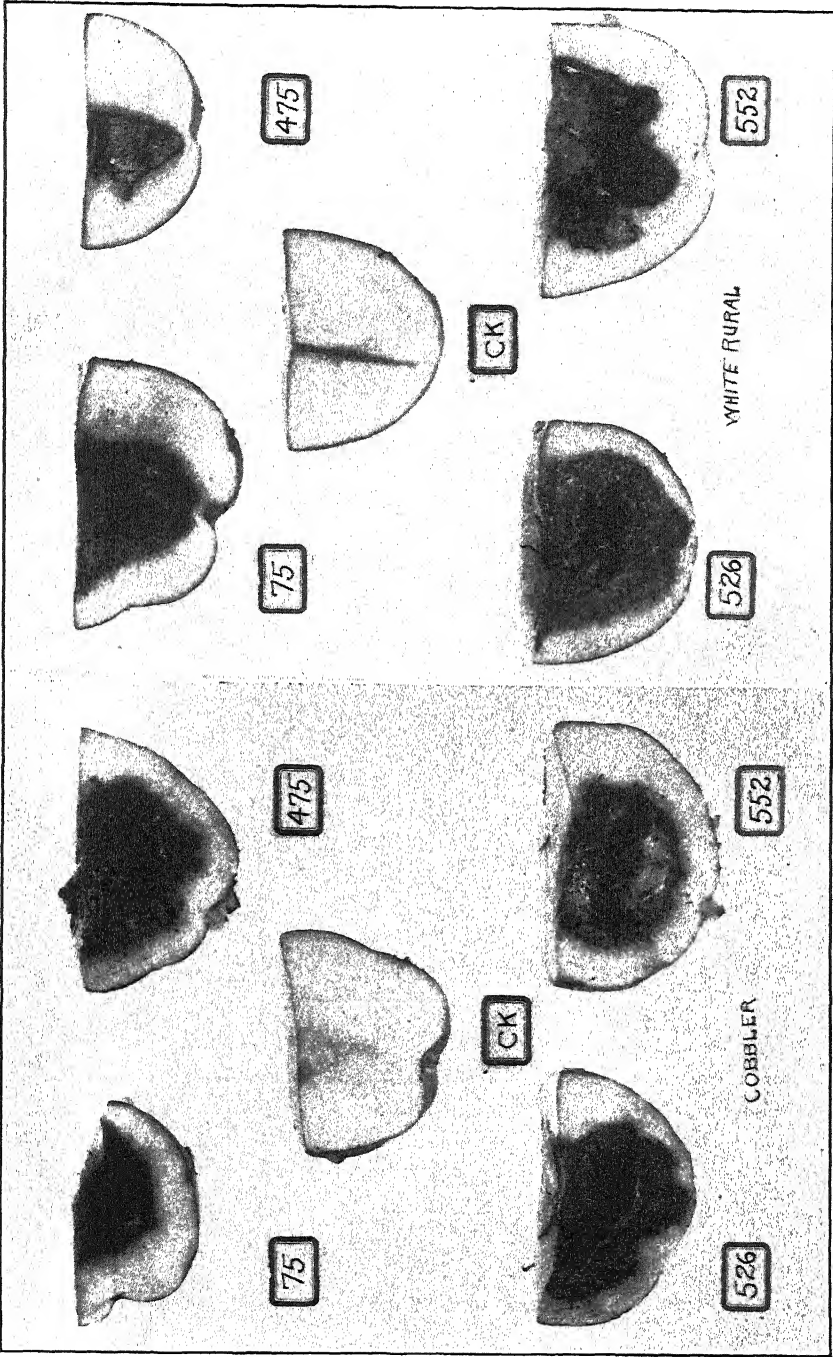
Reaction of potato varieties to bacterial rots. Inoculated January 5, photographed January 21, 1926. Incubated at room temperature. Left: White Rural; right: Irish Cobbler. CK. pricked with sterile needle, 75 *Bacillus mesentericus*, 475 *B. carotovorus*, 526 *B. phytophthorus*, 552 *B. aroideae*.

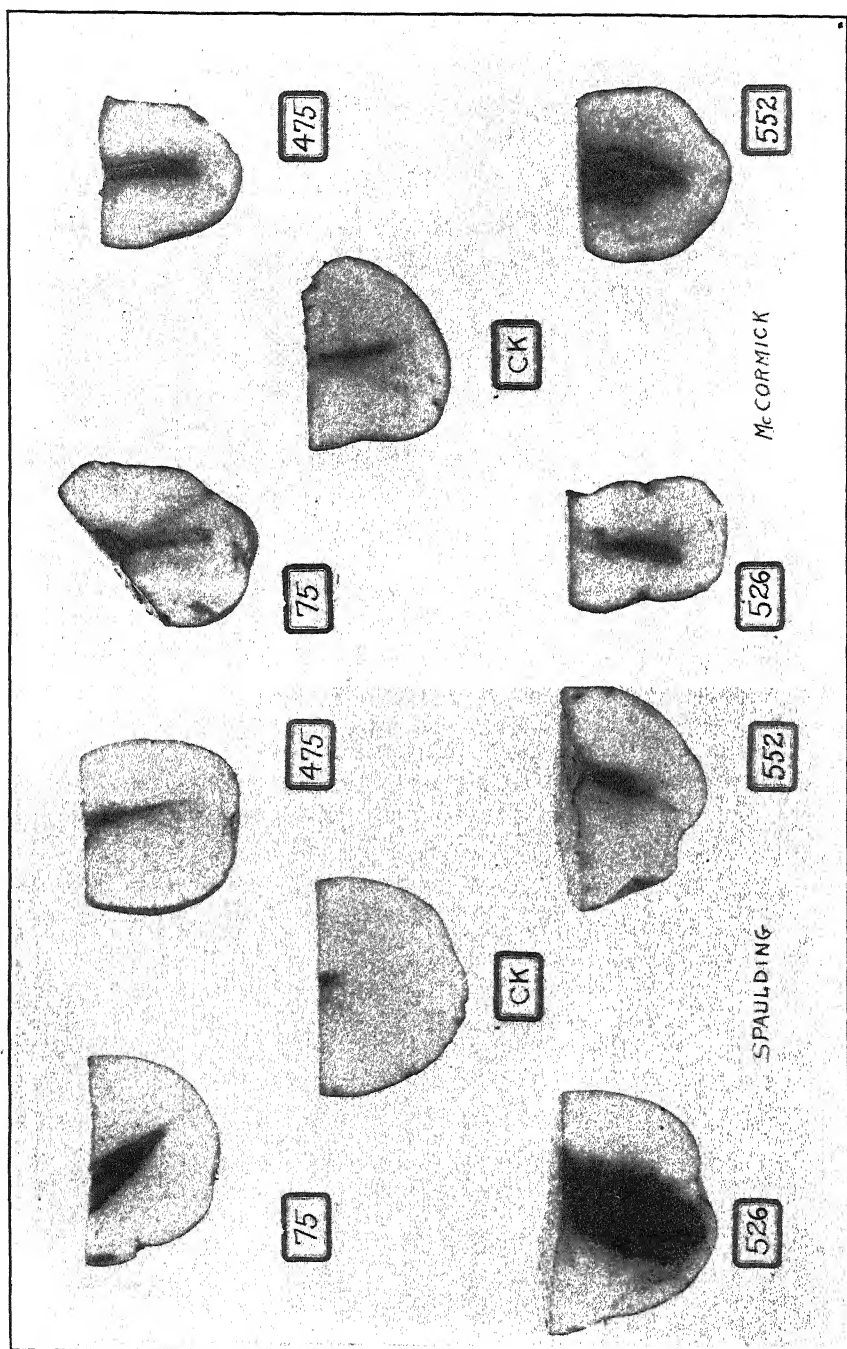
PLATE XXII.

Reaction of potato varieties to bacterial rots. Inoculated January 5, photographed January 21, 1926. Incubated at room temperature. Left: Spaulding Rose; right: McCormick. CK. pricked with sterile needle, 75 *Bacillus mesentericus*, 475 *B. carotovorus*, 526 *B. phytophthorus*, 552 *B. aroideae*.









PRELIMINARY STUDIES OF THE LIFE HISTORY OF *EROSTRO-
THECA MULTIFORMIS*, THE PERFECT STAGE OF
CLADOSPORIUM ALBUM DOWSON

G. HAMILTON MARTIN AND VERA K. CHARLES¹

In April, 1927, specimens of diseased sweet peas (*Lathyrus odoratus*) were received for determination by the Office of Mycology and Disease Survey from Dr. E. F. Guba, of the Massachusetts Experiment Station. Examination of the material showed the disease to be caused by a species of *Cladosporium* identical with *Cladosporium album* described by Dowson (4) in England in 1924. "White blight" is suggested by the authors for the name of the disease.

In May, 1927, the senior author made a preliminary survey for this disease in the greenhouses on Long Island, N. Y., and in northern New Jersey, where sweet peas were being grown. During this survey the disease was observed in only one greenhouse, that being on Long Island, although later in the year two more reports with specimens were received from New York.

Early in February, 1928, diseased sweet peas were received from a section in southeastern Pennsylvania. In all cases reported, the disease was the cause of considerable loss. One grower had an entire crop destroyed, while another grower who had been shipping to the New York City market for over 15 years said that unless a means of control was found he would have to stop growing sweet peas.

A critical examination of the Massachusetts, New York, and Pennsylvania collections showed the casual organisms to be identical with each other and also with the type material received from Mr. Dowson and deposited in the Mycological Collections of the Bureau of Plant Industry.

Cultures were made from the Massachusetts and New York material with the result that a perfect stage was developed from the New York collection. From this latter material only 1 out of 15 parts and vigorously growing cultures produced perithecia. The fact that Dowson failed to obtain a perfect stage and also that it was not developed from the Massachusetts material can best be explained by assuming the presence of strains. Certain phenomena exhibited in the course of the cultural work, which will be discussed in a later paper, also support this hypothesis.

The most remarkable character of the fungus under consideration is its polymorphism. In addition to seven distinct stages, a pseudo-sclerotial form was also observed. In certain of these forms the transitions from

¹ The writers are under obligation to Mr. M. L. F. Foubert for the photographic work illustrating this paper.

one stage to another can be easily understood and interpreted from a single slide. This is especially true in young or vigorously growing cultures. In old cultures the forms are found to adhere more closely to the types of the form genera that they represent. Conditions of temperature and humidity, and kind of media used are found to have a very great influence on the particular type of spore produced, but in this preliminary report no attempt will be made to correlate the character of growth with the physiological conditions under which the fungus has been grown.

At the present time the authors are unable to give a formula for the sequence in the development of the different spore forms, if indeed there is any law controlling such development. The object here, is to describe the different forms as they appear in nature and in pure cultures.

Considerable latitude may be permitted in definitely placing some of the conidial forms in the proper form genera. In view of the fact that these forms are all phases of the same species, the names used are more for convenience in describing them than for giving them a definite taxonomic position. The nomenclature followed is in most respects that of Briosi and Farneti (1) in their paper on white blight of lemon.

While the organism described in their paper is not identical with the present fungus, there are certain resemblances in the life histories of the two fungi which make their comparison of interest. A pycnidial stage was found by Briosi and Farneti and described as *Rhynchodiplodia*, but no ascospore stage was noted. On the contrary, in the study of the sweet pea fungus, no pycnidial stage was found but a perfect stage developed. The comparable conidial stages of the two fungi are as follows:

Rhynchodiplodia Briosi and
Farneti

CONIDIAL FORMS

Cladosporium citri
Hormodendron citri
Ovularia citri
Haplaria citri
Pseudofumago citri
Pseudosaccharomyces citri

PYCNIDIAL FORM

Rhynchodiplodia citri

SCLEROTIA

Not given

ASCOGENOUS FORM

Not given

Erothrotheca Martin and
Charles

CONIDIAL FORMS

Cladosporium album
Hormodendron cladosporioides
Ovularia form
Haplaria form
Pseudofumago form
Pseudosaccharomyces form

PYCNIDIAL FORM

Not found

SCLEROTIA

Pseudosclerotia

ASCOGENOUS FORM

Erothrotheca multiformis

DESCRIPTION OF THE DISEASE AND CAUSAL ORGANISM

The leaflets were covered with tan or buff-colored, circular and irregular-shaped spots varying from 2 to 20 mm. in diameter and often coalescing into large necrotic areas involving all or a large portion of the entire leaflet (Plate XXIII, A). The spots showed a tendency to be water-soaked about the margins. A close examination revealed numerous cinnamon-brown pustules, which imparted a granular appearance to the surface (Plate XXIII, B). On this infected area, numerous whitish tufts could be seen. These tufts consisted of conidiophores of the *Cladosporium-Hormodendron* form, and, when fresh, imparted to the spots a mealy appearance. In addition to this form, *Pseudofumago* and *Pseudosaccharomyces* were also present and became conspicuous when the leaves were allowed to remain in a moist chamber for 2 or 3 days.

The pseudosclerotia developed on the diseased areas in the course of a few days (Plate XXIII, C).

CULTURAL WORK

The inoculum for the culture work was all from the original culture of *Cladosporium album* in which the ascogenous stage developed. Accompanying morphological comparisons were made with the fungus on affected leaves received in February and March, 1928.

Various culture media were employed, but the larger part of the experimental work was done with cornmeal agar. It may be mentioned here that transfers from the same culture, when grown on potato and cornmeal agar, gave different types of growth. This applied not only to the production of conidial forms, but also to the development of perithecia, which were developed in abundance on cornmeal agar but not at all on potato agar.

For the sake of simplicity in treatment, the discussion will begin with the germination of the ascospore.

Ascospores (Plate XXIV, F) germinated (Plate XXIV, B and Plate XXV, A and B) readily at temperatures from 65° to 85° F. in about 18 hours. The growth was rapid and luxuriant, and conspicuously zonate. A *Haplaria* (Plate XXV, B and Plate XXVII, C) form was observed at the end of a few days. The *Cladosporium-Hormodendron* form of fructification followed in a short time. The *Cladosporium* consists of conidiophores bearing chains of 3-5 oblong, one- or two-celled hyaline spores (Plate XXIV, A, D). In old cultures the chains of spores were much longer, sometimes consisting of 10-12 spores. The usual form of *Hormodendron* appearing as a stage in the life history of this fungus and the only form observed by Dowson (4, p. 226) appears to differ from *Hormodendron cladosporioides* only in having larger hyaline conidia. In the course of these studies a dark form also developed from ascospore cultures.

The *Ovularia* form was observed at different periods in the growth of the cultures. In some cases it was found to develop from fine mycelium arising directly from the *Pseudofumago* and again it appeared in cultures of different ages. The spores are very small and often adhere to each other, forming an agglutinated head. The conidiophores are very erect, arise singly or in groups of 2 or 3, and measure about 28–35 μ in length. The spores are 1.5–2.5 \times 3.5–4.5 μ . The size of the spores is very dependent on the character of the medium.

The earliest stages in the development of perithecia were observed when the cultures were about six days old (Plate XXV A, Plate XXIV, A and B). They were formed abundantly and grew rapidly, maturing in about three weeks. (Plate XXIV, C). The irregularly biserial arrangement of the spores in the ascus may be seen at this time (Plate XXIV, E).

Pseudofumago occurred at different periods of growth and in association with the other spore forms. It consisted of coarse, irregular mycelium, composed of globular or ellipsoidal cells varying in size from 3–4 to 13–17 μ (Plate XXV, C). It was colorless when present in the tissues of the leaves. In culture the mycelium was hyaline, later becoming dark brown or greenish black. In this condition one or more cells may separate, becoming thick-walled and sometimes transversely divided. They are analogous to spores, capable of immediate germination or functioning as resting spores.

The *Pseudosaccharomyces* type of development was found to proceed directly from the *Pseudofumago*. This stage is so called on account of its resemblance to *Saccharomyces* and its manner of budding (Plate XXVI, A and B, and Plate XXVII, D). The various manifestations of growth exhibited during the transition of *Pseudofumago* and *Pseudosaccharomyces* are very numerous. *Pseudosaccharomyces* exhibits a remarkable polymorphism in its manner of budding and spore formation. Conidia may be borne in a whorl or crown at the apex of a terminal cell or on a protuberance which arises from any of the cells along the course of the mycelium. In advanced stages of the disease a pseudosclerotial form was observed on the leaves.

Forms of *Pseudosaccharomyces* are so similar to certain species of various form genera as to be indistinguishable from them morphologically. Among certain species included in this category may be mentioned *Protocoronospora nigricans* Atk. and Edgert. (10), *Kabatiella nigricans* (Atk. and Edgert.) Karak (6) on *Vicia*. All the budding conidial stages described and illustrated for this species correspond exactly with the species concerned in this investigation. It is of historical interest to note that in 1905 importations of *Vicia faba* originally from Egypt and Italy that were referred to this office were affected with apparently the same organism; in

this material, in addition to the budding form, several other stages comparable to those appearing in the present cultural work were noted. The fungus was not definitely determined at that time, it being two years previous to the publication of *Protocoronospora*. The similarity was confined to the conidial stages, no perfect form being found.

In April, 1927, various species of lilies growing in the vicinity of Washington were found to be affected with a disease that in morphological and cultural characters corresponds with the budding stages of the present fungus. Old cultures developed a *Pseudofumago* comparable to the *Pseudofumago* form discussed here. This fungus was identified as *Kabatiella*, probably *K. microsticta* Bub. described by Bubak (2) in 1907 as occurring on living leaves of the lily-of-the-valley in Bohemia. *Polyspora lini* (7) also presents comparable budding stages.

An attempt was made to place the perfect stage of the sweet pea fungus in a genus already established, but careful adherence to the generic description of nearly related genera made this impossible.

The fact that the spores are colored necessitated placing the genus in the group Phaeosporae of the Hypocreaceae.

In this section the genus *Melanospora* (3) is separated by the development of the beak and presence of dark spores. In *Neurospora* (8) the perithecia are described as subcoriaceous to subcarbonous, and the spores black when mature and longitudinally ribbed. A further separation is seen in the development of the perithecia. At no time in the development of the present fungus is the perithecial cavity filled with crowded septate hyphae such as are described for *Neurospora*. While the genus *Sphaeroderma* (5) is described as beakless, and the subiculum in some species is only poorly developed, it has several points of difference, among which are the longer-stiped, 4-spored asci; the spores, too, are characteristically darker than in the fungus described here. Even certain species of the genus *Sphaeroderma* described with 8-spored asci differ in other respects from the fungus described in this paper.

While there is a macroscopic resemblance to *Neocosmospora* (9) in the bright color of the perithecia and in the character of the walls, the genus differs in possessing brown, globose, verruculose spores and further by the absence of paraphyses.

Erostrotheca gen. nov.

Perithecia superficial, gregarious or scattered, globose-conical, beakless, glabrous, diaphanous; stroma absent; asci arising basally, evanescent, 8-spored; spores ellipsoid, yellow to olivaceous. (Perithecia superficial or embedded when grown in media.)

Erostrotheca multiformis sp. nov.

Perithecial stage; perithecia gregarious or scattered, characteristically zonate, when grown on cornmeal agar, at first Capucine yellow changing to mahogany red with age; asci clavate, $20-25 \times 12-16 \mu$, aparaphysate, short stipitate; ascospores irregularly biseriate, elliptical, flattened on one side, dark citrine, olive-yellow in mass. In agar culture.

Conidial stages: (*Cladosporium album* Dowson) spores elongate-cylindrical, continuous or 1-septate, $14-18 \times 3-4 \mu$; *Hormodendron* form, chains of spores simple or branched; spores pip-shaped to elliptical, both hyaline and colored in ascospore cultures. *Ovularia* form, conidiophores erect, arising singly or in groups of 2 or 3, $28-35 \mu$ in length; spores $1.5-2.5 \times 3.5-4.5 \mu$, often adhering to form agglutinated heads. *Haplaria* form, hyphae branched, hyaline, septate; conidiophores papillose or stipitate; conidia elliptical, continuous, hyaline, $6-8 \times 2-4 \mu$, forming agglutinated groups. *Pseudofumago* form, coarse, irregular mycelium, consisting of globular or irregular cells, $3-4 \times 13-17 \mu$. *Pseudosaccharomyces* form, cells saccharomycetous, hyaline, $8-16 \times 4-8 \mu$; conidia acrogenous or pleurogenous, spherical to oblong-elliptical. Pseudosclerotia minute brown to black on diseased areas of leaves.

Habitat: On living leaves and stems of *Lathyrus odoratus*.

SUMMARY

A recently recorded sweet pea disease in America caused by a *Cladosporium* is shown to be identical with *C. album* Dowson. This disease was described by Dowson in England in 1924 and recorded in America in 1927. "White blight" is suggested as the common name for this disease.

The perfect stage of *Cladosporium album* was developed in culture media and is described as *Erostrotheca multiformis*.

The fungus has many different spore forms, each of which is capable of starting a new infection. These are forms of *Cladosporium*, *Hormodendron*, *Haplaria*, *Ovularia*, *Pseudofumago*, and *Pseudosaccharomyces*. Pseudosclerotia are formed on the necrotic areas of leaves and are a means of spreading the disease.

Habitat: On leaves and stems of *Lathyrus odoratus*.

Known distribution: England, Massachusetts, New York, and Pennsylvania.

OFFICE OF MYCOLOGY AND DISEASE SURVEY,

BUREAU OF PLANT INDUSTRY,

WASHINGTON, D. C.

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A list of ten references is added for the use of workers especially interested in this subject.

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EXPLANATION OF PLATES

PLATE XXIII.

- A. Sweet pea leaflets showing spots caused by *Erostrotheca multiformis*.
- B. Portion of diseased leaflet showing granular appearance of spot. ×2.
- C. Pseudosclerotia formed on diseased leaflet. ×2.

PLATE XXIV.

- A. Conidiophore and spores of *Cladosporium*. $\times 400$.
- B. Germinating ascospores. $\times 400$.
- C. Mature perithecia. \times about 168.
- D. Young culture from a single ascospore showing conidiophore and spores of *Cladosporium* and young perithecium. $\times 400$.
- E. Ascus with spores. $\times 400$.
- F. Ascospores. $\times 400$.

PLATE XXV.

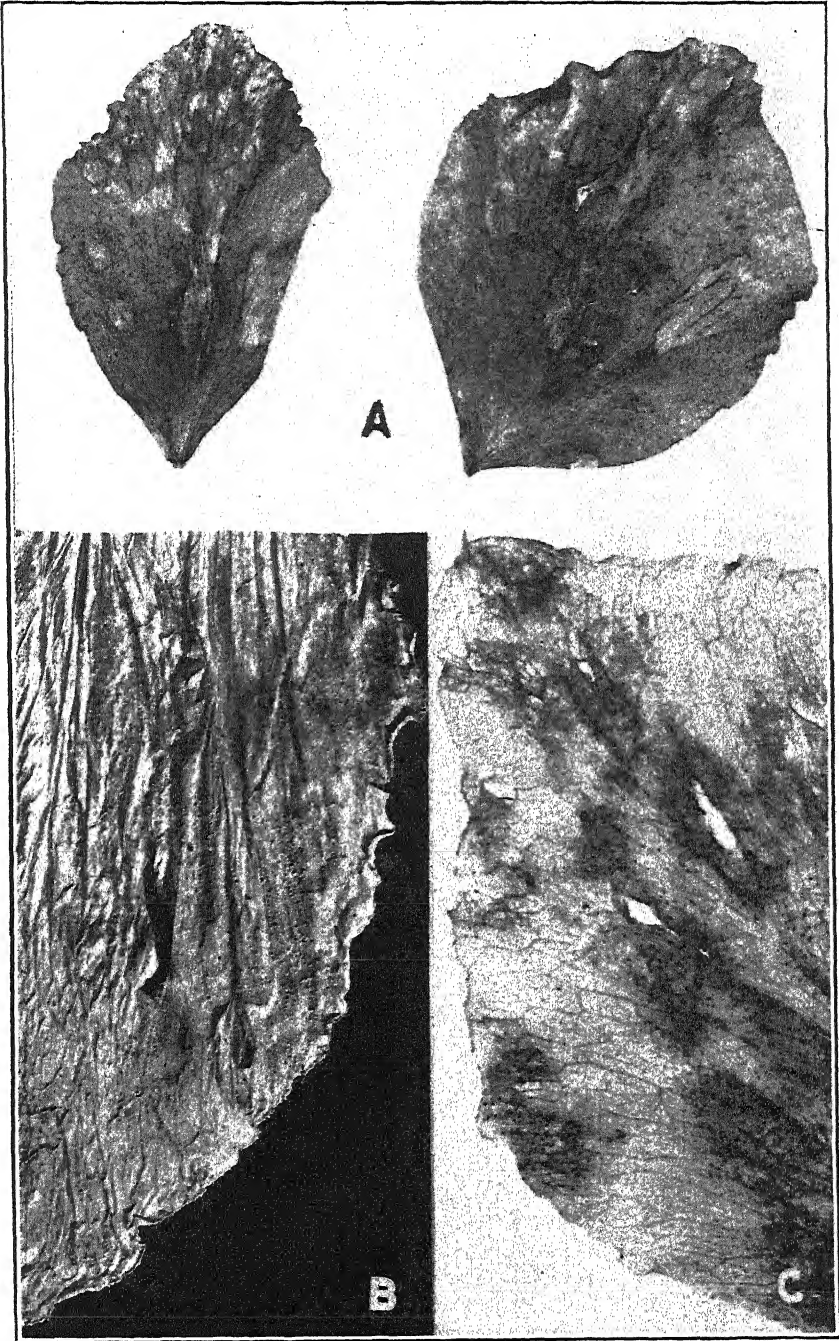
- A. Germinated ascospore and formation of young perithecia. $\times 400$.
- B. Germinated ascospore and formation of *Haplaria*. $\times 400$.
- C. *Pseudofumago*. $\times 400$.

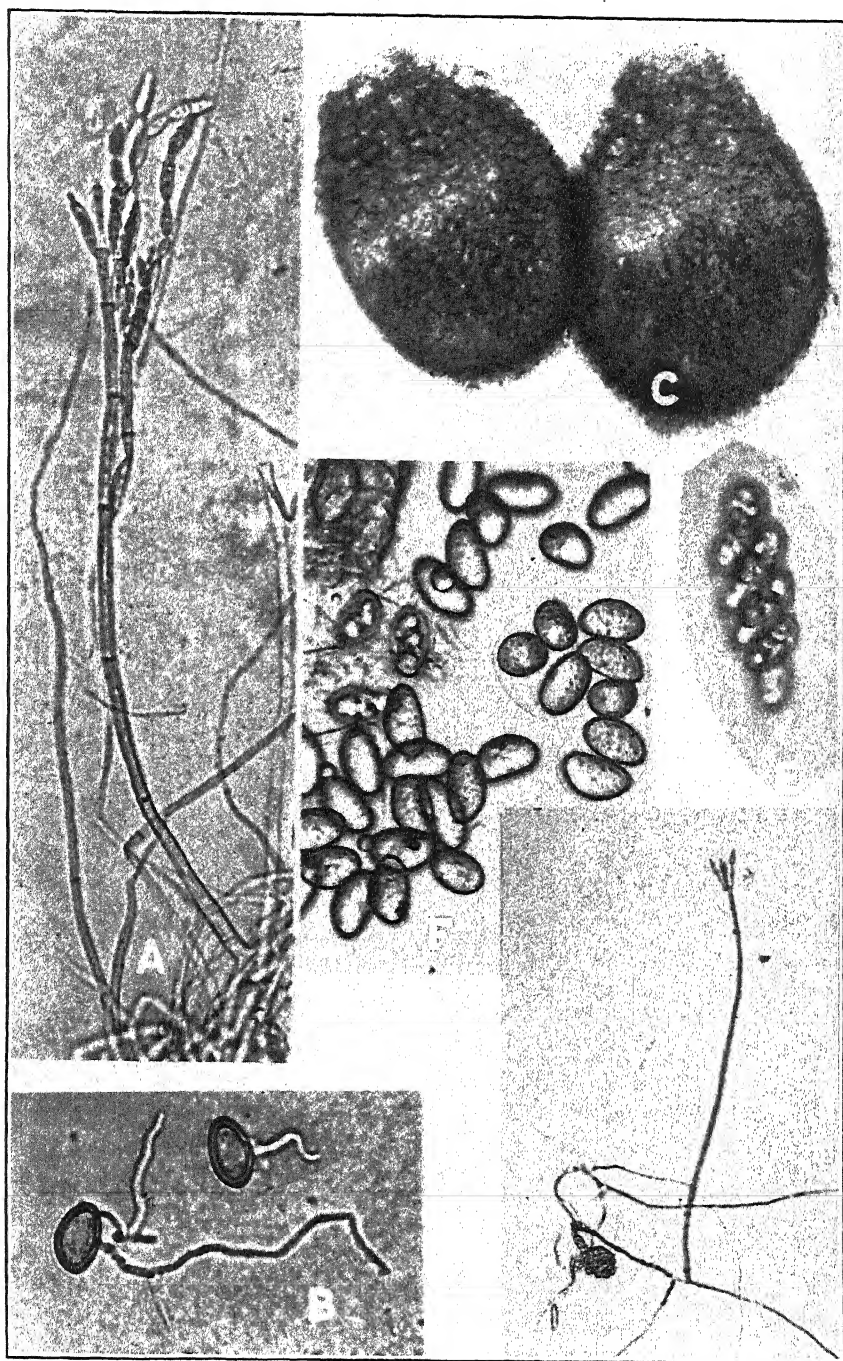
PLATE XXVI.

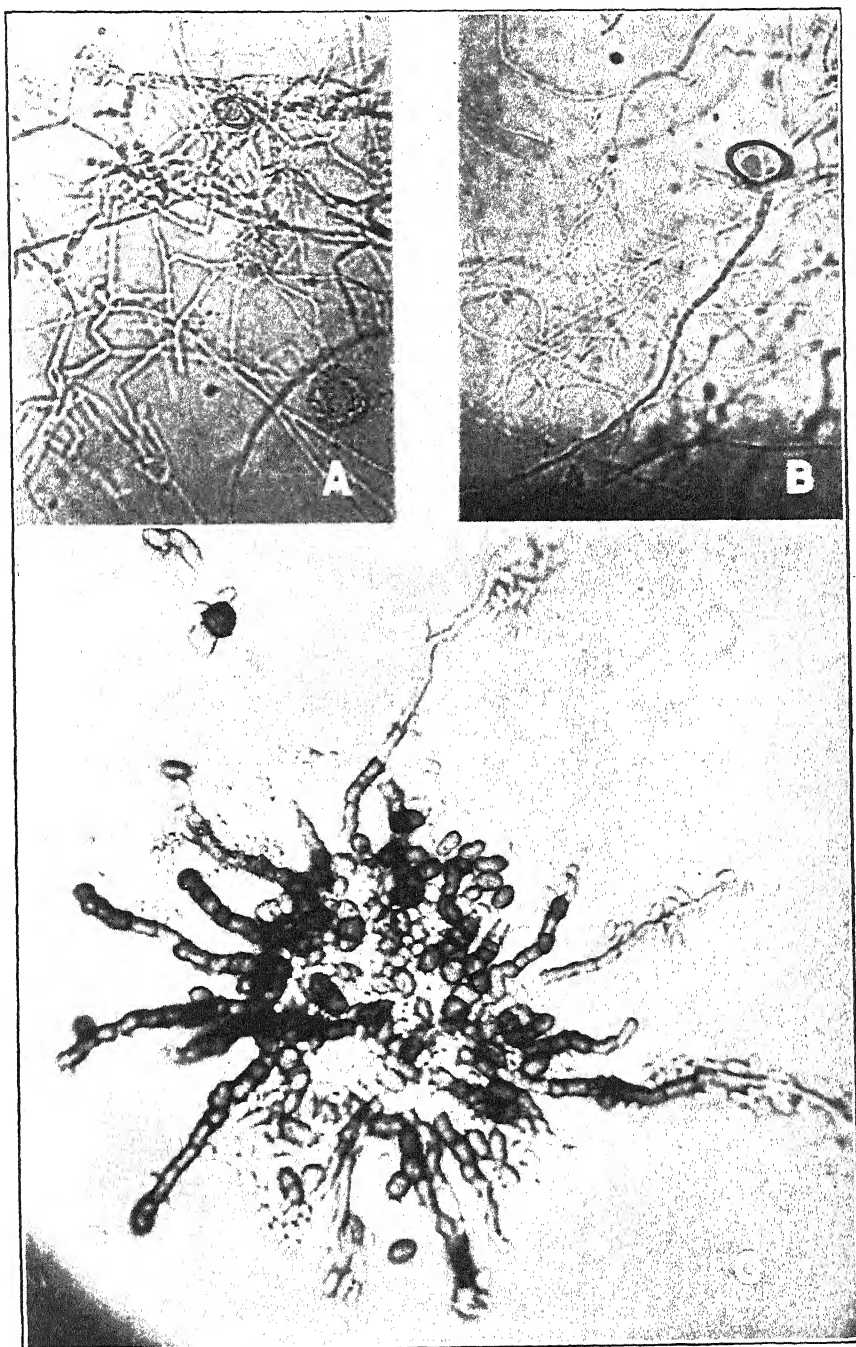
- A and B. Variations in *Pseudosaccharomyces* developed in culture media. $\times 400$.

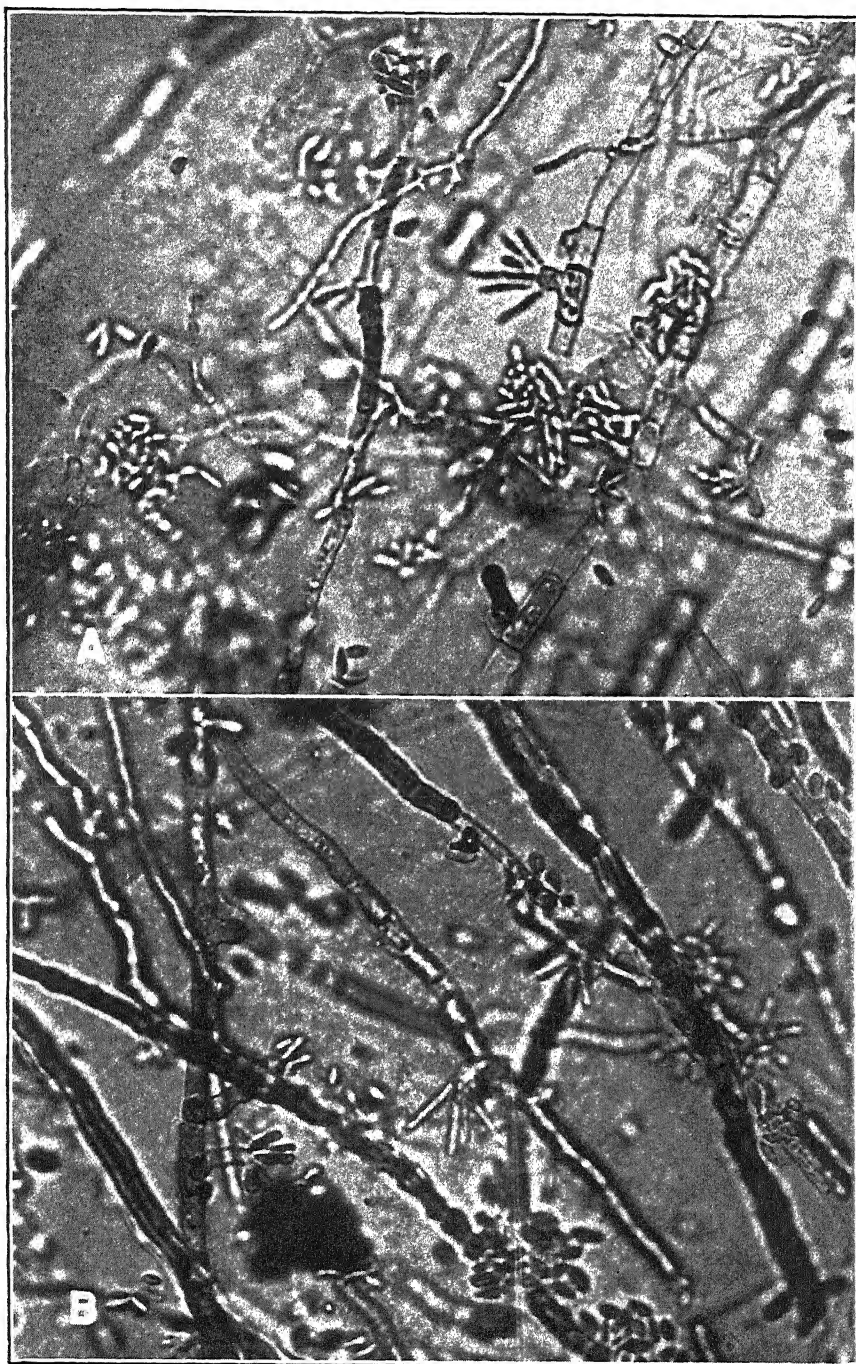
PLATE XXVII.

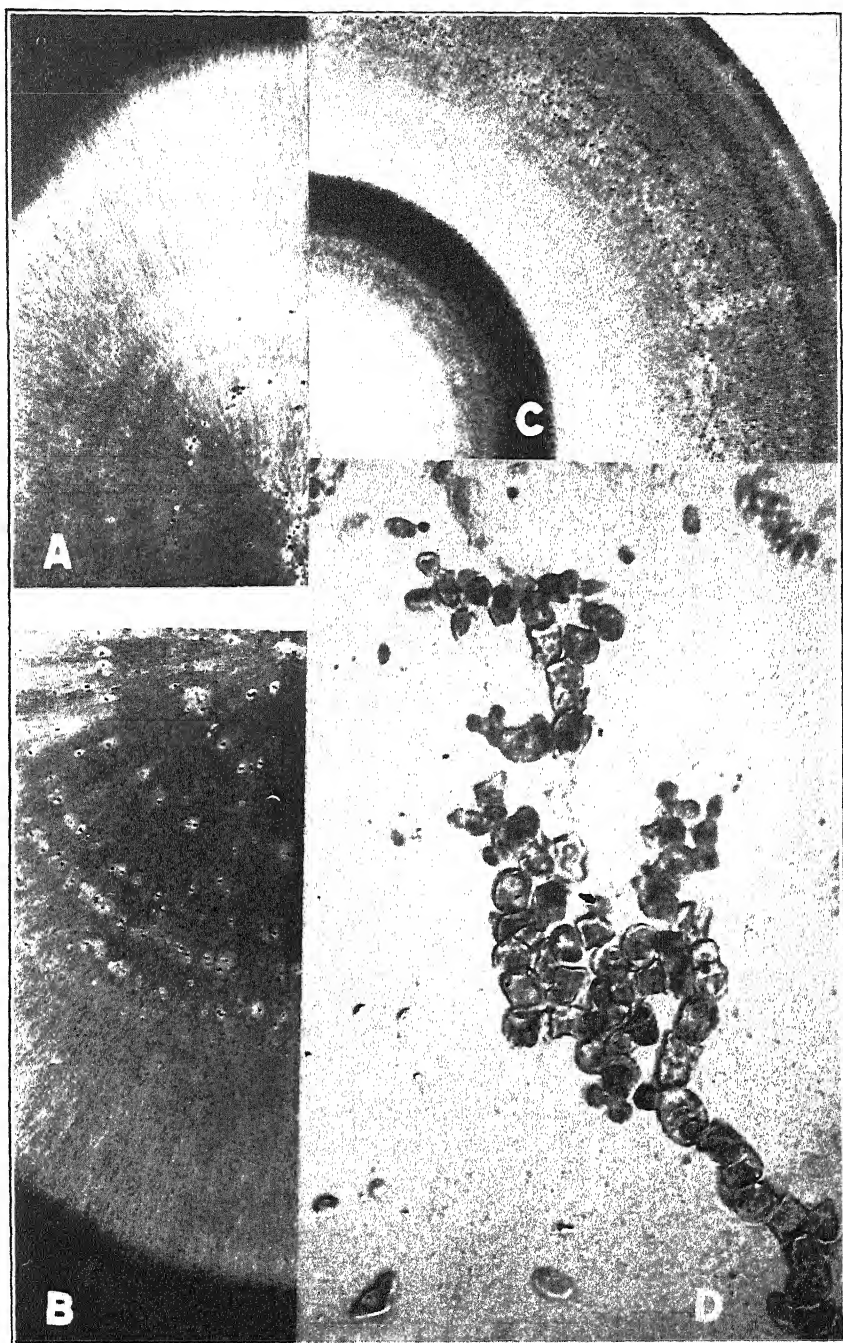
- A. Section of a petri dish culture from spores of *Cladosporium* showing formation of perithecia. $\times 2$.
- B. Section of a petri dish culture from a single ascospore showing formation of perithecia. $\times 2$.
- C. Section of a petri dish culture showing *Haplaria*, *Pseudofumago*, and *Pseudosaccharomyces*. $\times 2$.
- D. Germinating spores and hyphal development of *Pseudosaccharomyces*. $\times 400$.











CONTROL OF CUCUMBER POWDERY MILDEW IN GREENHOUSES¹

E. F. GUBA

INTRODUCTION

Powdery mildew, caused by the fungus *Erysiphe cichoracearum* DC., is a common disease of greenhouse cucumbers in Massachusetts. Epidemics occur between May and October when little or no heating is practiced and during the colder months in periods of dull weather when heat is not required to maintain the minimum temperature of 60° F. for growing cucumbers. When moisture-saturated-air conditions are not prevented by heat, powdery mildew becomes epidemic. The control of this disease by regulation of the greenhouse air in the absence of pipe heat has not proved possible. The fungus yields readily to control with fungicides. Their nature, the method and rate of application under varying conditions, and their compatibility with insecticides, the use of which is also necessary, have not received previous study. Guba (3) reported the incompatibility of copper fungicides and hydrocyanic acid gas in greenhouses. This fact has prohibited the use of hydrocyanic acid gas, and tobacco fumigants have been substituted.

Both copper and sulfur fungicides are used for combating powdery mildews. Sulfur preparations are more satisfactory chiefly because sulfur offers a more lasting protection from infection and is toxic in the absence of moisture. Warm temperatures, however, are an important factor with sulfur but not with copper.

The delicate nature of greenhouse cucumber foliage and the high temperatures and bright rays of the sun to which it is subjected at times require certain precautions in connection with the choice and use of fungicides to avoid injury.

Under greenhouse conditions dusting is the most practical method of control. There are times, however, when mildew becomes prevalent on the lower surfaces of leaves where fungicidal dusts cannot be deposited. The protected position of the fungus is a handicap to its successful eradication with sulfur dusting materials during periods of cool, dull weather. Disinfection of the foliage with liquids toxic to mildew and safe for the foliage would be the logical practice to follow in such cases.

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With the discovery by Whitcomb (15) of the merits of Volek, a commercial, highly refined, white lubricating oil emulsion, for controlling the Greenhouse red spider (*Tetranychus telarius* Linn.) on Greenhouse cucumbers, this oil spray has come into extensive use for this purpose. The use of Volek for red spider and of sulfur for mildew, however, is attended with injury. Since Volek is the most satisfactory insecticide known for combating red spider, and since powdery mildew and red spider frequently are present on the cucumber concurrently, there has arisen the need of fungicides the use of which would be compatible with this oil spray.

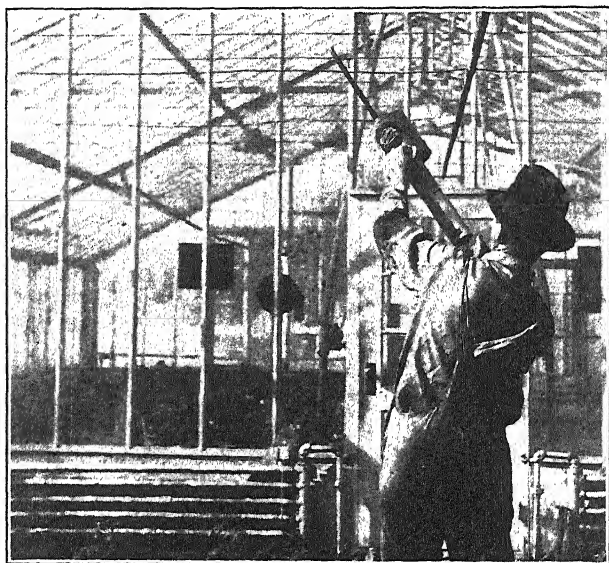


FIG. 1.—Type of hand duster most suitable for use in greenhouses showing proper method of operation for controlling cucumber powdery mildew.

CONTROL EXPERIMENTS WITH DUSTING MATERIALS

Materials Used and Methods of Application

During the period 1925–1928 inclusive, greenhouse cucumbers were dusted with various brands of sulfur. The treatments were made when powdery mildew was generally prevalent in the houses. The materials used were Grape Dust, Slug Shot, Tricked Sulfur, Sulfur-Gypsum, and Anchor Sublimed Velvet Flowers of Sulfur. Slug Shot contains 6 per cent free sulfur, Grape Dust 64 per cent, Tricked Sulfur 91 per cent, and Anchor Brand practically 100 per cent. The Sulfur-Gypsum dust was prepared to contain 15 and 20 per cent free sulfur and 80 and 85 per cent gypsum, both of 200-mesh fineness.

The Feeney Model D 2-quart duster holding $2\frac{1}{2}$ pounds of dust was employed in the work. The use of this piece of equipment proved very practical in greenhouses (Fig. 1). The wires, posts, pipes, and plants offer no interference to its operation, and discharges of dust are easily obtained. Hand dusters which are strapped to the body are impractical in greenhouses.

Previous to dusting, the ventilators were closed to prevent the dust from being carried outside by air currents. Cloudy days, evenings and mornings are the best periods for dusting during the warm months of the year since at such times the ventilators can be closed long enough to permit the dust to settle without subjecting the plants to unfavorable temperatures.

The dusting of the greenhouse air was done from the walks and between the rows of vines according to the construction of the house. In houses 40 feet wide provided with walks in the center, on the sides and ends, all of the dusting was done from the walks (Fig. 2, a). In houses more than 25

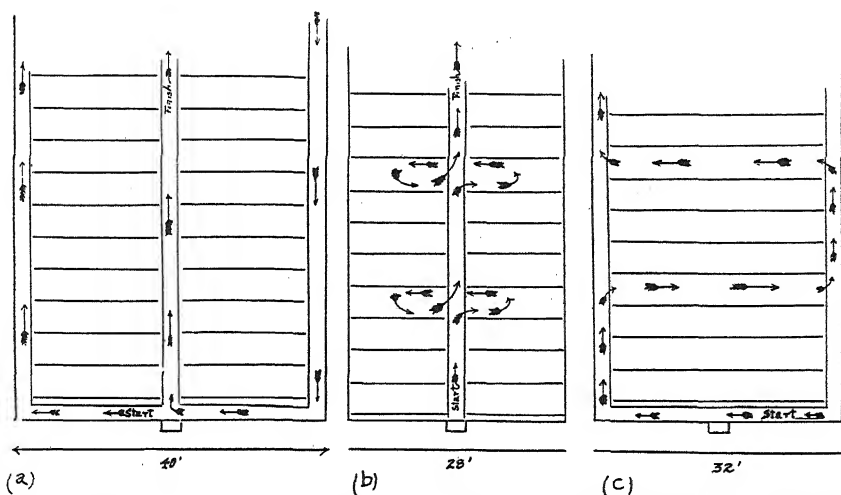


FIG. 2.—Course of travel in greenhouses of different types with duster for controlling cucumber powdery mildew.

feet wide provided only with a central walk, every 3 to 5 rows of plants were entered for about one-half the distance (Fig. 2, b). In houses with walks only around the bed, every 3 to 5 rows of plants were traversed (Fig. 2, c). At every 5 to 10 paces the discharge tube of the duster was projected above the wires overhead. One to two pushes of the plunger with the duster in an obliquely vertical position provided sufficient dust for about 500 square feet. The occasional prevalence of mildew on the lower sheltered leaves, especially where the vines were grown over the wires, re-

quired horizontal discharges of dust to destroy it. Five to six pounds of dust were used in one application in the largest houses of the dimensions 280×40 feet and where mildew was generally prevalent. Smaller quantities were used in smaller houses of where mildew was localized.

Discussion

Grape Dust, Tricked Sulfur, Sulfur-Gypsum, Anchor Brand Sulfur Dust, and Slug Shot proved excellent for dusting purposes. These materials are fluffy and fine, but Slug Shot was not so effective as the other grades. In sunlight and high temperatures characteristic of the warmer months Slug Shot has been effective in the control of mildew but not in cooler weather. The small quantity of sulfur in this material is not fungicidal under mild sunlight and temperature conditions even when in contact with mildew. Under such conditions good control has been obtained with the other sulfur dusts used.

Observations and experience have taught that heavy applications of sulfur dusting materials cause injury. Since the sulfur-gypsum preparations containing 15 and 20 per cent free sulfur have given control equal to that from dusting materials much higher in sulfur content it has also become apparent that dusting materials generally in use for combating powdery mildew contain more than the amount of free sulfur necessary to give control. The use of these high-sulfur-content dusts and excessive doses of such materials have been the chief factors contributing to injury.

Small doses of dust are extremely desirable on greenhouse cucumber plants. For houses of the dimensions 280×40 feet irrespective of volume, not more than 5 to 6 pounds of dust should be applied at one time. Since mildew usually appears first either at the doors or near the ventilators, there is no need of using the maximum quantity upon the first appearance of the disease. The dust should be used sparingly to avoid hardening of the leaves, and dusting should be started when mildew makes its appearance rather than after the fungus is generally established. The dust should be discharged into the air above the vines and permitted to settle before the ventilators are opened.

VAPORIZED SULFUR

The vaporization of sulfur has been practiced many years in greenhouses. Application of sulfur preparations to the heating pipes and the use of mechanical sulfur vaporizers (Plate XXVIII) for controlling powdery mildews have been reported by Bailey (1), Humphrey (6), Maynard (8), Höstermann (5), Norton and White (11), Norton (10), Rupprecht (12), Vogt (13, 14) and Barker and Wallace (2). Sulfuring of the heating

pipes gives control when the pipes are well heated, and is considered an effective method. Most growers have not taken kindly to the practice because the treatment corrodes the pipes, interferes with radiation, and does not always give satisfactory control. Mechanical sulfur vaporizers have lacked practical application largely because danger of losing the crop from burning sulfur has been associated with their use.

The lack of adaptation to practice of the earlier types of mechanical sulfur vaporizers led to study and use of equipment devoid of their dangerous and impractical features. The equipment that has proved most satisfactory for greenhouses consists of electric hot plates, requiring about 4.5 amperes each, porcelain evaporating dishes, asbestos board, and electric wire of No. 6 or 8 guage (Plate XXVIII e, f, g). It is operated on a 110-volt circuit and with a meter box having amperage capacity for the number of plates used. A more complete description of the apparatus and its method of operation will be presented in a paper on the control of the leaf-mold disease of greenhouse tomatoes.

The equipment was operated in large commercial rose and cucumber houses and in the range of the Market Garden Field Station at Waltham, Massachusetts. Powdery mildew has been controlled repeatedly by a single operation of the equipment, but the completeness of disinfection was associated with prevailing bright, warm weather. An extremely thin deposit of sulfur on the foliage, hardly perceptible to the naked eye, has given complete control. The strong odor of sulfur in the houses following the treatment and most pronounced on bright, warm days was a distinct feature of this method of sulfuring.

During the months of January and February, 1926, experiments were conducted on a small scale to determine the relation of the rate of application to control. Cucumber vines growing in ground beds in the greenhouse were enclosed in glass chambers of 175 cubic feet and treated with vaporized sulfur varying in rates from $1\frac{1}{2}$ to 9 pounds for each 100,000 cubic feet. The temperature during the time of vaporization varied from 65° to 75° F. During the period between treatment and final observations, the temperature of the greenhouse reached 80 to 90° on five days and 75 to 80° on three other days. Equal control of epiphyllous infection was obtained by all doses, but in each case at this season of the year mildew on the sheltered foliage and lower leaf surfaces was not eradicated.

The control experiments with vaporized sulfur demonstrated the marked susceptibility of powdery mildew in contact with small doses of sulfur, and that, under continued dull or damp weather conditions, sheltered surfaces escape disinfection. The results are in line with the generally established fact that high temperatures and sunshine contribute to

the activity of sulfur at a distance. In bright warm weather vaporized sulfur has given complete disinfection of the foliage. Under such conditions the atmosphere of the greenhouse acquires a strong odor of sulfur. The action of dusting sulfur is similar but the distribution of sulfur by its vaporization is more uniform than dusting. It gives a more complete disinfection of the houses with less sulfur and the quantity of sulfur vaporized is easily regulated. After the equipment is once permanently installed its operation is convenient, but sulfur vaporization requires more time than dusting. The initial cost of equipment as well as later expense of replacing dishes and heating units is also to be considered. The extremely fine dusting sulfurs now available and the ease with which they are applied hardly justify the vaporization of sulfur on the hot pipes or with mechanical apparatus; neither would the practice be so profitable a means of control for commercial establishments as dusting. If vaporized sulfur were the most effective and practical means of obtaining control, as has been demonstrated for tomato leaf mold, caused by *Cladosporium fulvum* Cke., the practice would be warranted.

CONTROL EXPERIMENTS WITH LIQUID FUNGICIDES

Under dull or damp weather conditions the spread of powdery mildew on lower and sheltered leaf surfaces cannot be suppressed with sulfur dusting materials. Serious attacks of powdery mildew confined to lower leaf surfaces have been noted in commercial greenhouses which could not be controlled with sulfur dust. Experiments were conducted to determine the merits of liquid fungicides under such conditions.

Volck as a Fungicide

McWhorter (9) reported control of rose mildew, *Sphaerotheca pannosa* (Wallr.) Lévl., with Volck. As far as the writer is aware this is the only published report of the fungicidal efficiency of this oil spray. Three treatments of Volck (1 pt. to 4 gals. of water) were applied at intervals of two to three weeks. The season's control of rose mildew from these treatments was considered remarkable.

The writer used Volck in a series of tests in the greenhouse on mildewed cucumber foliage. The 1 per cent strength recommended for controlling red spider was used. At this strength, the oil spray occasionally was somewhat toxic to mildew. Instances have been noted of toxicity only in the centers of mildewed areas, while the margins remained perfectly healthy. Usually treatment with the oil spray was followed within 24-48 hours after application by the appearance of numerous new infections, which indicated that the spray was directly responsible for this spread. In some

instances total elimination of the fungus was observed but there was no protection from subsequent infection. Lack of control was outstanding. The value of Volck in respect to its fungicidal activity to powdery mildew of cucumbers is not significant. Its use alone for red spider when powdery mildew is also present cannot be considered as fulfilling the requirements for control of the latter.

Copper Sprays

Bordeaux mixture is fungicidal to powdery mildew. Tests with 1-1-100, 2-2-100, and 4-4-100 mixtures were made. The first two mixtures left no objectionable residue on the fruit and foliage and the third left a heavy residue and a hardened foliage. All mixtures have consistently given equal control. Certain dry and liquid copper fungicides diluted to contain the same amount of metallic copper in 1-1-100 Bordeaux spray, *i.e.*, 0.03 per cent, proved equally active against mildew. Those considered were Basic Copper Sulfate prepared by Holland *et al.* (4) containing 26 per cent copper, Hammond's Copper Solution 3.05 per cent, Dow Powdered Bordow 12.5 per cent, and Grasselli Bordeaux Mixture Powder 13 per cent.

Copper sprays are necessary when atmospheric conditions render sulfur dusting materials ineffective, but contact of the spray with mildew is necessary to obtain eradication. The use of fine, up-turned nozzles and high pressures are essential to insure wetting of the lower sides of the leaves.

Copper fungicides do not offer protection from infection. This fact was clearly demonstrated in an experiment in which cucumber vines were sprayed on August 26 with bordeaux mixtures, 1-1-100 and 2-2-100, with and without saponin added to assist wetting. Equal control was obtained in each case, but mildew appeared again, necessitating the use of sulfur on September 6, eleven days after the copper application. Similarly, 20-80 monohydrated copper sulfate-lime dust is not toxic to mildew on dry foliage. The same dust gives control if the foliage is syringed with water immediately before or after dusting. Lack of protection from infection with copper spray residues is due to the fact that the fungus develops on the foliage in the absence of moisture, which is essential to copper activity. Copper sprays, however, are extremely useful during cool, dull weather when sulfur offers no control.

Sulfur Sprays

Commercial liquid lime-sulfur concentrate, potassium sulfide, and dry mix sulfur lime were considered. The tests were made under bright weather

conditions in September, 1927, and April, 1928, at temperatures above 85° and 90° F. Lime-sulfur concentrate was used at dilutions ranging from 1-200 to 1-1000. Below the dilution of 1-200, burning and a conspicuous residue resulted and the foliage acquired a hardened texture. Dilutions from 1-200 to 1-1000 were fungicidal, but the stronger of these left an objectionable residue on the cucumber. Potassium sulfide at stronger than 1-400 dilutions caused burning. This fungicide at 1-400 to 1-1000 dilutions was fungicidal to mildew, caused no injury and left no perceptible residue. Dry mix sulfur lime sprays of 2, 3 and 4 per cent suspensions proved safe but their residues on the fruit render this material undesirable.

Potassium sulfide, 1 pound to 50 gallons of water, may be used in place of bordeaux 1-1-50 for combating lower leaf surface infection. It gives slightly better protection than bordeaux, altho the choice of either type of spray should be influenced by its compatibility with insecticides recommended for controlling insects and red spider.

COMBINATION TREATMENTS

Copper Sprays and Volck

The absence of control with Volck and the possibility of increasing the prevalence of powdery mildew rather than suppressing it led to consideration of a combination of the oil spray with copper fungicides. The advantage of a combination spray which would control red spider and powdery mildew with one treatment was obvious.

It was found that acid solutions and Volck were incompatible. The former separated the oil from the emulsion. For this reason weak dilutions of copper sulphate and sulfuric acid could not be considered. All copper fungicides considered gave alkaline reactions in water and produced compatible mixtures with this oil spray. These were Holland's Basic Copper Sulfate, Hammond's Copper Solution, Dow Powdered Bordow, Grasselli Bordeaux Mixture Powder, and freshly made bordeaux mixture 1-1-100. Temperatures of 84-86° F. prevailed at the time of application with lower temperatures not below 50° F. during evaporation of the spray. All of these combinations with Volck gave equal control but Volck alone gave little or none.

In another experiment, plats of cucumber vines were treated on August 1 with Volck 1 per cent, Volck 1 per cent and bordeaux 1-1-100 combined, and bordeaux 1-1-100. The foliage sprayed with the oil emulsion alone showed, a few days after application, numerous new centers of infection, and mildew had by August 8 assumed such prevalence that bordeaux 1-1-100 was applied to suppress it. The application of bordeaux on August 1 gave excellent

control and the foliage remained free of mildew until August 12, but further treatment was not considered necessary until August 18 (Fig. 3). Volck and bordeaux 1-1-100 combined and bordeaux 1-1-100 alone proved equally effective as disinfectants. The combination spray spread much better than

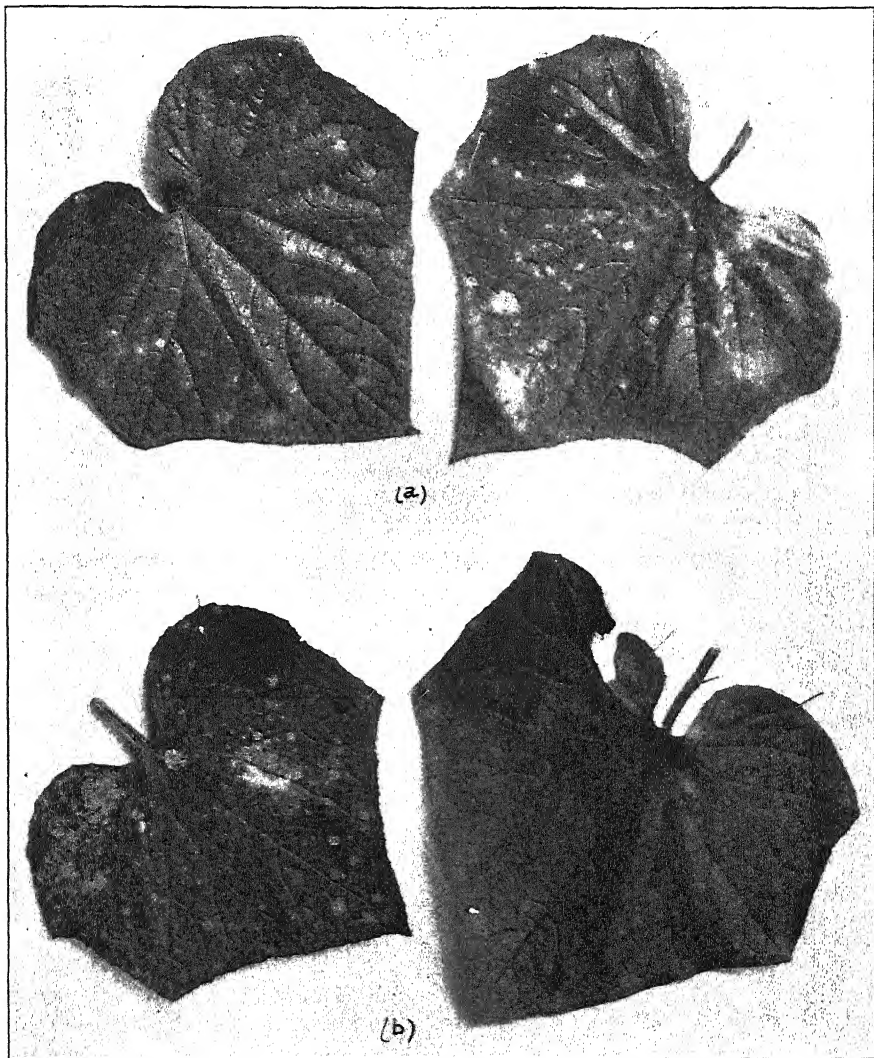


FIG. 3.—Cucumber leaves. (a) At left treated with bordeaux 1-1-100 containing 1 gallon of Volck concentrate. At right treated with Volck of the same strength. (b) At right sprayed with bordeaux 1-1-100, at left no treatment. Photographs six days after application.

TABLE 1.—*Toxicity of copper fungicides to powdery mildew and injury to foliage following fumigation with hydrocyanic acid gas*

Materials	Copper con- tent of con- centrate (per cent)	Rate of dilution	Toxicity to foliage	Toxicity to mildew	Injury with hydro- cyanic acid gas
Basic Copper Sulfate	26.00	0.96 lb.-100 gals.	-	+	Moderate
Hammond's Copper Solution	3.05	8.19 " -100 "	-	+	Severe
Dow Powdered Bordow	12.50	2.00 " -100 "	-	+	do
Home-made bordeaux	25.00	1-1-100	-	+	do
Grasselli Bordeaux Mixture	13.00	1.91 lb.-100 "	-	+	do
Water			-	-	None
Volek Spray 1 per cent			-	-	do
Volek and Basic Copper Sulfate	26.00	0.96 " -100 "	-	+	Slight
Volek and Hammond's Copper Solution	3.05	8.19 " -100 "	-	+	Moderate
Volek and Dow Powdered Bordow	12.50	2.00 " -100 "	-	+	Slight
Volek and Grasselli Bordeaux Mixture Powder	13.00	1.91 " -100 "	-	+	Severe
Volek and home-made bordeaux	25.00	1-1-100	-	+	Slight

Volek. Since complete wetting of infected foliage is essential to total disinfection, this improvement in the wetting of the oil spray resulting from the addition of bordeaux mixture was considered a distinct advantage.

Copper Sprays and Hydrocyanic Acid Gas

According to Guba (3) the use of hydrocyanic acid gas in the greenhouse when copper deposits are present on foliage is attended with severe foliage burning. Foliage sprayed with bordeaux mixture 1-1-100 and later exposed to hydrocyanic acid gas was severely burned. Injury also occurred from using 0.04 per cent copper sulphate solution on tomato plants when subjected to the gas.

Rows of cucumber plants were sprayed with different brands of copper fungicides alone and in combination with Volek 1 per cent, and diluted on the basis of copper contained in 1-1-100 bordeaux spray, *i.e.*, 0.03 per cent metallic copper. One week after application the greenhouse was fumigated with hydrocyanic acid gas generated from calcium cyanide. The latter was used at the rate of $\frac{1}{4}$ ounce to each 1,000 cubic feet. When copper sprays were used in combination with the oil spray, injury was slight with two exceptions (Table 1). When copper sprays alone were used injury was severe and of about the same degree. The oil emulsion apparently provides some protection to the copper residue from being acted upon by the fumigant. Injury is serious enough to make inadvisable the use of hydrocyanic acid gas when the combination is used. Tobacco fumigants or sprays should be substituted in such cases.

Sulfur Fungicides and Volek

Sulfur fungicides and Volek are not compatible. In the majority of cases called to the writer's attention injury occurred from using Volek after sulfuring. Injury was also observed in one establishment where the oil was used three times on the same crop in the absence of sulfur. Injury is manifested in the form of yellowing of the leaf accompanied by burning. The foliage appears oily and glossy and when dried is semi-transparent, resembling parchment. Finally the leaves turn brown and crisp. Manifestation of injury is rather slow and apparently related to the slow rate of penetration of the oil.

Young cucumber plants were treated with Volek and Grape Dust, Tricked Sulfur, and Slug Shot. Injury occurred in either order of application of oil emulsion with Grape Dust and Tricked Sulfur, but none with Slug Shot. Plants treated with these dusting materials or with oil emul-

sion alone were not injured. In further experiments sulfur dust containing 15 and 20 per cent free sulfur have proved compatible with this oil spray. Inferentially the compatibility of Slug Shot with Volek is due to its small sulfur content. Since 15 and 20 per cent sulfur dusts are fungicidal to the fungus, sulfur dusts of higher contents are not necessary. High grade sulfur dusts should not be risked on cucumber vines that have been, or are to be, sprayed with Volek. If the use of the oil spray for red spider is an established necessity, low grade sulfur dusts should be used to combat mildew. Dusting materials containing 15 and 20 per cent free sulfur are of much greater merit than Slug Shot and can be prepared at a great saving in cost.

In further experiments sulfur spray materials were compared for their compatibility with Volek. Half rows of plants were sprayed with 0.5 and 1 per cent lime-sulfur, 0.13 per cent potassium sulfide, and 5 and 12 per cent dry-mix sulfur lime. Three days later the entire rows were sprayed with 1 per cent Volek. Lime-sulfur 1 per cent caused burning, but the other sulfur sprays none. Following the oil emulsion treatment the foliage of the sulfured vines gradually turned yellow. Injury was most severe in the plats receiving 1 per cent lime-sulfur and dry-mix sulfur lime. The foliage on the other half of each row, which was sprayed with oil emulsion only, remained healthy. Nine days after the oil emulsion application these half-rows which received only the oil spray were sprayed with sulfur fungicides corresponding to treatments given the other half of the rows. Severe burning appeared in the plat treated with 1 per cent lime-sulfur and more or less yellowing and burning resulted from the other treatments, except from potassium sulfide 0.13 per cent. Injury was more pronounced when the oil spray followed sulfur than where the order of application was reversed. This is in line with experiences growers have had in using sulfur and Volek.

During bright weather of April, 1928, and prevailing high greenhouse temperatures vines were sprayed with a 2 per cent suspension of dry-mix sulfur lime, 0.25 per cent potassium sulfide, 0.5 per cent lime-sulfur, and 1 per cent Volek. Two vines in each plat received the 1 per cent Volek treatment in addition to the sulfur sprays. Vines receiving both the oil emulsion and sulfur treatments showed yellow foliage and burning which gradually became very serious. The results have shown that sulfur sprays can not be used on greenhouse cucumbers which require the oil spray for combating red spider.

SUMMARY

Powdery mildew on cucumbers in greenhouses is readily controlled with fungicides, but their choice requires caution to avoid dangerous combina-

tions with insecticides, and their strength and rate of application require modification to prevent injury.

Sulfur fungicides are most effective for combating cucumber powdery mildew in greenhouses. Slug Shot, containing 6 per cent free sulfur, is fungicidal only in bright weather and high temperatures. Sulfur dusting materials containing 15 and 20 per cent free sulfur and high grade dusts containing 64, 91, and 100 per cent free sulfur have given equal control. The latter should be used sparingly to avoid injury. Low grade sulfur dusts are safer than high grade sulfur dusts, and therefore are preferable.

Applications of sulfur dust should not exceed 5-6 pounds in houses 280 × 40 ft. Smaller quantities should be used in smaller houses or when mildew is localized.

In cool or damp weather sulfur dusting materials are not effective and do not control lower leaf surface infection. Under such conditions copper or sulfur sprays have proved effective.

Bordeaux 1-1-50 is preferable to stronger mixtures. The latter harden the foliage and leave residue on the fruit. Proprietary copper fungicides containing an equivalent amount of copper are fungicidal. Disinfection is obtained by wetting.

Commercial lime-sulfur concentrate is safe in dilutions from 1-200 to 1-1000 and potassium sulfide in 1-400 to 1-1000. Stronger dilutions are toxic to the foliage. Lime-sulfur 1-200 and potassium sulfide 1-400 provide slightly better protection than copper sprays. Potassium sulfide does not leave a noticeable residue, and therefore is preferable to lime-sulfur.

Highly refined, white lubricating oil emulsion (Volek) exhibits mild fungicidal activity to mildew but does not control it.

Sulfur fungicides and Volek are not compatible. High grade sulfur dusts, 0.5 per cent lime-sulfur, 0.25 per cent potassium sulfide and 2 per cent dry-mix sulfur lime sprays caused injury when used before or after the oil spray. Slug Shot and low grade sulfur dusts containing 15 and 20 per cent free sulfur are compatible.

Volek may be combined with bordeaux or with proprietary copper fungicides. Bordeaux 1-1-50 or proprietary materials diluted to contain an equivalent amount of copper are recommended. The mixture is recommended if mildew is present when the oil spray is required for red spider.

Copper fungicides and hydrocyanic acid gas are incompatible. Fumigation with hydrocyanic acid gas should not be practiced if copper residues are present on the foliage. Tobacco sprays or fumigants should be used in such cases.

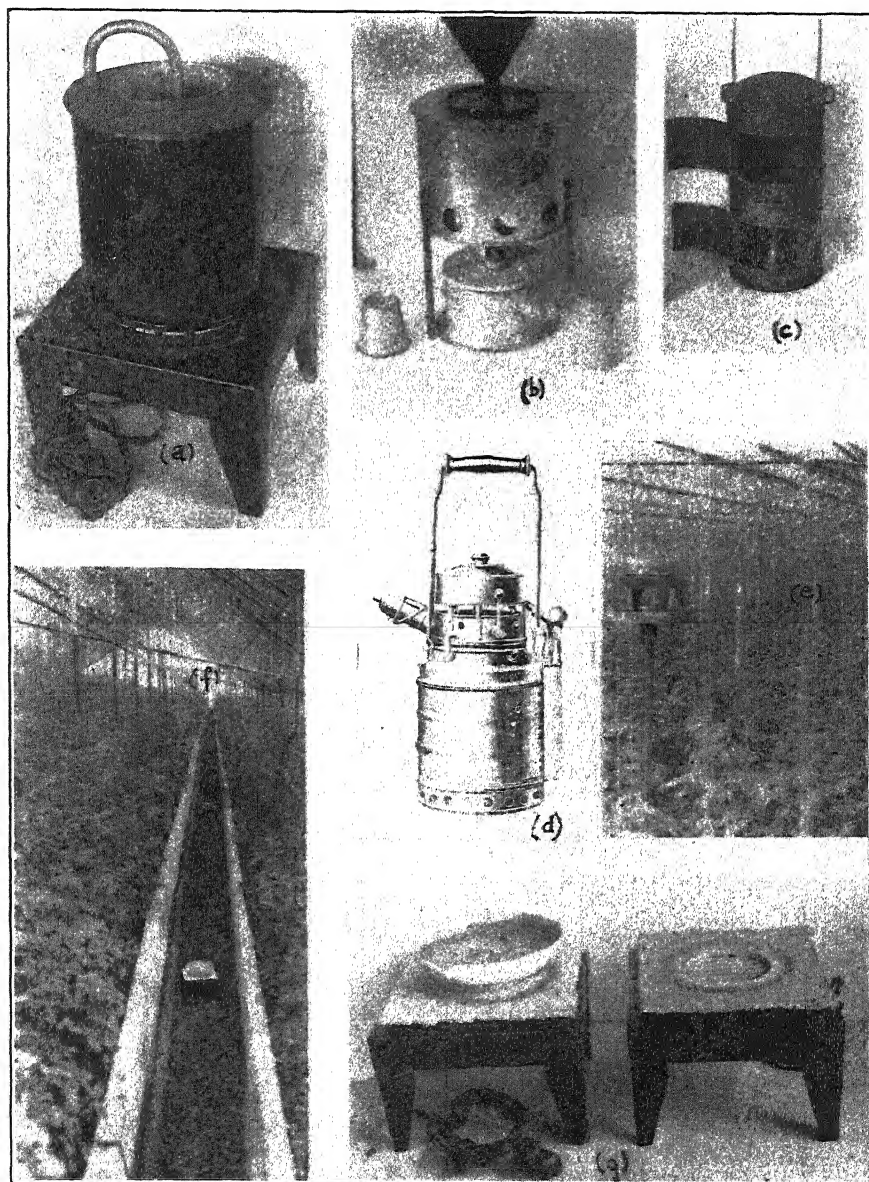
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EXPLANATION OF PLATE XXVIII.

(a), Sulfur Sublimier; (b), Campbell's Patent Sulfur Vaporizer; (c) Dorrance Sulfur Burner; (d), Rota-Generator; (e), Vaporizing equipment assembled in tomato house; (f), Vaporizing equipment assembled in rose house; (g), Electric hot plate, asbestos board, and porcelain evaporating dish assembled for operation.



THE ROOT-KNOT OF ABACÁ, OR MANILA HEMP¹

GERARDO OFFIMARIA OCFEMIA AND MELANIO R. CALINISAN

THE DISEASE

Root-knot is a very common, though not usually serious, disease of abacá, or Manila hemp (*Musa textilis* Née) in the Philippine Islands. In severe cases and in advanced stages of the disease the roots of the plants turn brown, die, and rot. On account of the death of all, or of the greater part of, the roots, infected abacá plants become much stunted, their leaves become small and pale green or yellowish, and the petioles may be more or less bunched up at the upper end of the pseudo-stem. Owing perhaps to the common occurrence of root-knot on abacá in Cavite and Laguna provinces, where the bunchy-top disease is most destructive, Hernandez (5), Teodoro (10) and Teodoro and Serrano (11 and 12), all of the Philippine Bureau of Agriculture, believe that bunchy-top is caused in part, at least, by *Heterodera radiculicola*. Fahmy (3) in Egypt describes a disease of banana, a relative of the Manila hemp, which is due to a *Heterodera* different from *H. radiculicola*. This author states that the disease causes dwarfing of the banana and the production of small upright leaves which are arranged in the form of a rosette. Fahmy (3) reports that when more than 50 per cent of the roots of the banana are affected the rosette condition is distinct and many of the roots are rotted. The disease is also said to cause the pseudo-stem to split, and later the inner tissues rot. According to Magee (6) the Egyptian banana trouble is identical with the bunchy-top disease in Australia.

The present paper reports the results obtained in experiments conducted from 1925 to date which show that the nematode occurring in the galls of abacá roots and in the soil in fields where the bunchy-top disease occurs, causes a malady entirely different from the bunchy-top (7 and 8).

Symptoms

On aërial parts.—The indication on the aërial parts of the plant that abacá is infected by root-knot may be noted on the leaves. They become

¹ Mr. Calinisan used part of the data herein reported in a thesis presented by him for graduation with the degree of Bachelor of Agriculture from the College of Agriculture, University of the Philippines, March, 1928. He was responsible for experiments carried on from April, 1927, to January, 1928, and for the study of the nematode.

Contribution from the Experiment Station of the College of Agriculture at Los Baños, Laguna, Philippine Islands. Published with the approval of the Director.

pale green to yellowish and this paleness is more pronounced in the youngest leaf. At first not much difference may be seen between healthy (Fig. 1, A) and infected plants (Fig. 1, B) except in size. As the disease advances, however, the leaves gradually become shorter and narrower than those of healthy plants of the same age. The plant becomes stunted and the leaves tend to crowd at the upper end of the pseudo-stem. The symptoms exhibited by the aerial parts of the abacá, however, are not reliable because other conditions, such as poverty of the soil, or lack of available moisture in it, crowded planting of abacá seedlings, and mechanical injuries to the roots may cause exactly the same changes.

On the underground portions.—The most reliable symptom of root-knot is the presence of the galls in the roots (Fig. 2, B). The galls are of varying shapes and sizes, depending upon their location on the roots. They may be from 3 to 10 or more mm. in diameter. Several of these galls may run together so that the infected portion of the roots may appear as an irregular, almost club-shaped body, sometimes 5 cm. long by 1 or more



FIG. 1. Abacá seedlings, 3 months and 22 days old, of variety Sinibuyas showing the relative size of plants grown in sterilized nematode-infected soil (A) and unsterilized nematode-infected soil (B) after two months and 17 days. Note that the plants in B, though much stunted, do not show symptoms of bunchy-top. (Photograph by the photographic division, department of agronomy, College of Agriculture, Los Baños, P. I.)

cm. in thickness. At first the roots are normal in color, or almost yellowish white, but later they become brown to almost black. The surface of the galls becomes rough with age on account of the cracking of the epidermis and cortex. In advanced stages the roots die so rapidly that new ones are developed, but these are finally infected and killed.

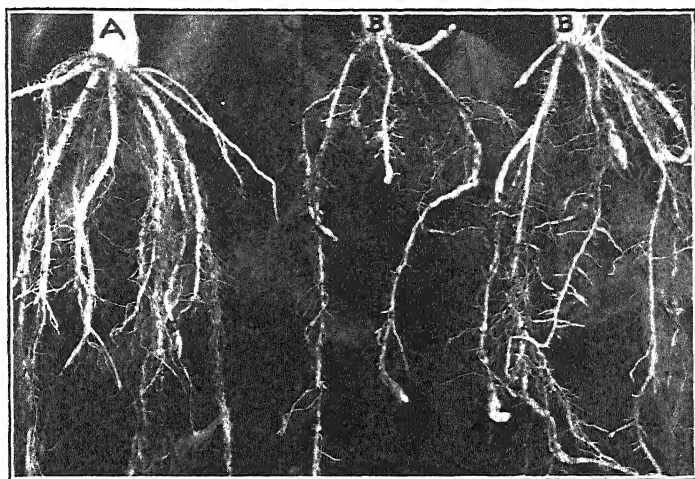


FIG. 2. Three root systems of the abacá seedlings shown in figure 1, A and B. At the left (A) is the root system of one of the plants in the sterilized infected soil after allowing the plants to grow in it for two months and 17 days. The middle and right root systems (B) are those of plants in the unsterilized infected soil showing almost club-shaped roots due to galls produced by *Heterodera radiculicola*. (Photograph by the photographic division, department of agronomy, College of Agriculture, Los Baños, P. I.)

CAUSAL ORGANISM

Egg.—The egg of *Heterodera radiculicola* is almost elliptical, or cylindrical with rounded ends. According to Byars (2) the eggs are about $88 \times 35 \mu$. Godfrey (4) reports that usually the egg of *H. radiculicola* is less than $1/250$ inch, or about 100μ in length. Measurements made at Los Baños of 50 eggs of the abacá root-gall nematode showed that the size range is $76-108 \times 36-48 \mu$. The average size of the eggs is $91.8 \times 41.3 \mu$, or 3.8μ longer and 6.3μ thicker than those reported by Byars (2). The embryo ranges from $80-112 \times 36-48 \mu$. The average size is $93.9 \times 41.1 \mu$.

Larva.—Godfrey (4) states that the length of a newly hatched larva of *H. radiculicola* is about $1/80$ to $1/50$ of an inch, or about 312.5 to 500μ , and the diameter of the body is about $1/30$ of its length or about 10.4 to 16.6μ . According to Byars (2) the larvae are about 400μ in length. Fifty larvae

of the male abacá-root nematode were measured and the size range of these was $331.6\text{--}464.2 \times 16.5\text{--}25.9 \mu$. The average size is $409.5 \times 18.8 \mu$. Compared with Godfrey's (4) figures the larvae of the abacá nematode have an average length which lies within the range given by him and an average thickness which is 2.2μ greater. The larva molts several times, but no work was done to determine the number of molts before maturity is reached.

Measurements of 50 female larvae show that the size range is $352\text{--}547.1 \times 32\text{--}91.1 \mu$. The average size is $432.3 \times 50.7 \mu$. The size range of the female nematode approaching maturity is $416.7\text{--}769.4 \times 144.3\text{--}432.8 \mu$. The average size is $514.7 \times 269.1 \mu$.

Male.—The male abacá nematode is long, of a typical cylindrical shape, and tapering at both ends. Baylis and Daubney (1) state that the body of the worm is covered with cuticle which is striated, but it has no bristles. The head of the male is distinct, and there are six lips. The stylet is composed of three rods, which are fused throughout and knobbed behind. It has two testicles. The posterior end has no alae or papillae. The spicules are equal, short, and broad. Godfrey (4) found that the mature male nematode measures from $1/20$ to $1/16$ of an inch in length, or about $1,250$ to $1,562.5 \mu$, but not over $1/40$ as thick as it is long, or about 31.25 to 39.06μ . Byars (2) reports that the mature male is a little more than 1 mm. long. Scofield (9) states that the size of the male nematode is from 1 to 1.5 mm. long.

Female.—The body of a mature female abacá nematode becomes swollen into an ovoid or pear-shaped structure. Only the neck portion of the worm remains normal. According to Baylis and Daubney (1), in the female nematode there is a terminal prominence which takes the place of the tail and this carries the vulva. The anus becomes located on the dorsal side. Baylis and Daubney (1) further state that *Heterodera radiculicola* is oviparous. The eggs, however, remain in the uterus. After the death of the female the eggs hatch and the larvae then escape.

In regard to size Byars (2) states that the greatest diameter of the mature female *Heterodera radiculicola* is about 0.5 mm., or 500μ . Godfrey (4) reports that the diameter of the female nematode is from $1/40$ to $1/25$ of an inch, or about from 625 to 1000μ . According to Scofield (9) the mature female nematode is less than 1 mm., but oftentimes it is about $3/4$ of a millimeter, or 750μ in length. The writers found that the size range of the mature female nematode of the abacá is $720\text{--}1280 \times 400\text{--}800 \mu$. The average size is $1056.1 \times 619.5 \mu$.

The size of the eggs of the abacá nematode in the Philippines is nearly the same as that of Byars (2) and Godfrey (4) for the eggs of *H. radiculicola*. The characteristics of the male and female nematode agree with

those given by Baylis and Daubney (1) for *Heterodera*. The presence of two testicles in the mature male shows that it is *H. radiculicola*. In order to confirm the writers' determination, specimens were submitted to Marcos A. Tubangui of the Department of Veterinary Parasitology, College of Veterinary Science, Los Baños, Laguna; he identified them as *H. radiculicola*.

THE RELATION OF HETERODERA RADICICOLA TO ABACÁ, OR MANILA HEMP AND TO
THE BUNCHY-TOP DISEASE

It was first proved by the senior author (8) in August, 1925, that the bunchy-top of abacá in the Philippines is caused by virus which is transmitted from diseased plants to healthy abacá by the aphid *Pentalonia nigronervosa* Coq. Experiments were conducted to determine if bunchy-top could be transmitted by planting healthy abacá seedlings in soil which was collected from areas infected with bunchy-top in Laguna and Cavite provinces. The soil in the bunchy-top-infected areas of Laguna and Cavite and the roots of the infected abacá contain numerous nematodes. On account of the constant association of nematodes with roots of abacá infected with bunchy-top, these eelworms have been suspected of being the cause of bunchy-top (5, 10, 11, and 12). In the experiments which are reported in this paper, 24-centimeter pots, or 5-gallon kerosene cans which were cut into halves, were filled with the nematode-infected soil taken from Laguna and Cavite. One of the pots or cans of soil was always sterilized for a check in the autoclave for two hours at 15-pounds' pressure. The pots were planted with three to five seedlings of abacá which were grown from seeds. After planting the seedlings the pots were placed on a wooden bench out of doors where they were watered at regular intervals.

After from 38 to 51 days it was noted that the checks were much taller and darker colored than the plants in the unsterilized soil (Fig. 1, A and B). All of the plants were then dug up and their roots were carefully examined. It was always noted that galls of various sizes were present on the roots of all plants grown in the unsterilized soil, while the roots of the checks were clean and healthy (Fig. 2, A and B). In all cases the symptoms of the plants whose roots were infected with nematodes were different from those of plants with bunchy-top.

Further inoculations under controlled conditions were carried on by the junior author in 1927. Inoculations were made (a) by planting abacá seedlings grown from seeds in soils very heavily infected with *Heterodera radiculicola* taken from bunchy-top-infected areas in Cavite; (b) by pouring around the roots of the seedlings, which were growing in sterilized soil, a suspension of from 25 to 100 nematode larvae obtained by allowing the eggs to hatch in sterile Pfeffer agar (2); and (c) by mixing with sterilized

garden soil, before abacá seedlings were planted, a quantity of chopped abacá roots which had galls in them.

The results of all of the experiments made in 1927 were exactly the same as those obtained by the senior author from 1925 to 1926. In all cases the symptoms produced on the abacá were those of root-knot, a disease which is entirely different from bunchy-top.

CONCLUSIONS

From the results of the inoculation experiments the writers conclude that the nematode *Heterodera radiculicola* produces root-knot on the abacá. This disease occurs on a wide range of hosts in the tropics as well as in temperate countries. The disease is localized in the roots. Although the symptoms of root-knot are also manifest on the aerial parts of abacá, nevertheless these are very different from those of bunchy-top, which is a systemic disease of the type of the mosaics and related chloroses. There is therefore no evidence justifying the connecting of *Heterodera radiculicola* with bunchy-top as heretofore reported by Hernandez (5), Teodoro (10), and Teodoro and Serrano (11 and 12).

SUMMARY

Root-knot is a very common disease of abacá, or Manila hemp, in the Philippine Islands. The disease is not usually serious. On account of the wide distribution of root-knot in abacá districts where bunchy-top occurs, the two diseases were regarded as synonymous.

Root-knot, due to *Heterodera radiculicola*, causes dwarfing of abacá plants, yellowing of the foliage, reduction in size of the leaves, and formation of galls in the roots. The roots finally die and rot. The disease is entirely different from bunchy-top, showing that the nematode cannot cause this disease as heretofore reported.

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DIFFERENTIAL STAINING OF PERONOSPORACEAE¹

E. LEPIK

In the cytological work of plant pathology the differential staining of the parasites in the interior of the host is an important operation. The mycelium must form a distinct contrast to the surrounding host tissue, so that both of them can be clearly differential from each other and the pathogenic agent easily demonstrated. The success, however, is not always the same; the usual staining processes in cytology cannot be applied in every case.

In those cases in which the mycelium is distributed in the woody parts, e.g., *Melampsorella caryophyllacearum* in *Abies pectinata*, the differential staining can easily be obtained with a chloride-zinc-iodine reaction. In the case of *Cronartium ribicola* in white-pine Colley (1) uses 1 per cent safranin and 1 per cent lichtgruen dissolved in 95 per cent alcohol. With Ascomycetes Vaughan (7) successfully arrived at the usual Planeze (6) method in the cytology of higher plants.

With Peronosporaceae various staining methods have been frequently checked, whereby differential staining, too, was successful in some special cases. Mangin (4) uses benzidine dyes: benzol blue with benzolazurine and rosazurine in the alkaline bath, or Orselline BB with aniline blue in the acid bath. In the former case the hyphae are red and the host cells blue; in the latter case the reverse is true. Mangin's formula was subsequently tested by Wartenweiler (8), but he was unable to obtain differential staining on account of the poor quality of the dyes at his disposal. Otherwise he uses for the mycelium of *Plasmopara nivea* a mixture of Bavarian blue and orange, or Flemming's threefold staining. Differential stainings are not obtained thereby, only distinct pictures.

Klebahn and Philipp (5) were able to obtain good pictures in the case of *Peronospora pulveracea* Fuck. with congored, by first freeing the object from the protoplasm with Eau de Javelle. This process, however, cannot well be applied to microtome cuttings, because our present adhesives do not convey the Eau de Javelle, and segregate the cuttings from the glass.

According to Kobel (3) Uredineae and Peronosporaeae can be stained with a solution of 0.1 gr. of aniline blue, 50 cc. of concentrated lactic acid and 100 cc. of water. The cuttings are left in this for five minutes, then rinsed in water, and warmed in a drop of lactic acid on the glass. The

¹ This work has been carried out with the support of the International Education Board, under the direction of Professor Dr. E. Gäumann.

mycelium takes up the stain intensely, while the host remains almost colorless. This simple process is favorable both for fresh and for herbarium material and can be applied at the same time to hand and microtome cuttings. It cannot, however, be used for permanent preparations.

When staining Peronosporaceae "bleu coton" is often used; according to Kobel (3) and Jacques Pottier this process probably originates with Prillieux. J. Pottier calls a mixture of "bleu coton GHB" and lactic acid "bleu lactique."

Klebahn² first stains the cuttings with "bleu coton GBBBB" in lactophenol (1 part water to 1 part glycerine, to 1 part phenol, to 1 part lactic acid) and afterwards with Orange G in oil of cloves. With this solution he obtains fine contrasts of blue and yellow.

Finally, good staining may likewise be effected in the case of Peronosporaceae with Haidenhain's haematoxyline. In objects that have been carefully differentiated with ferric alum the mycelium is bluish and the surrounding tissues are blackish. In those mycelia in which closer study of the nuclear conditions is intended, the haematoxyline method is even to be preferred to all others.

STAINING WITH BLEU COTON AND SAFRANIN³

Cuttings which have been obtained in the usual way by the microtome and attached to the glass by albuminous glycerine are freed from paraffin in oil of turpentine or xylol. The latter is dissipated with absolute alcohol and rinsed for a short time with 96 per cent alcohol. The glass with the cuttings is then placed in a flask with lactophenol-alcohol (I). The lactophenol⁴ is composed as follows:

(I) Phenol (carbolic acid) free of water.....	10 gr.
Lactic acid, concentrated	10 cc.
Glycerine, thickened	20 cc.
Alcohol, 96 per cent.....	20 cc.

After 15 minutes the cuttings are taken from the lactophenol-alcohol and placed in the following mixture:

² For the particular information about his method of staining I am indebted to Professor H. Klebahn, Hamburg.

³ The stains were supplied by the firm of Dr. Bender and Dr. Hohbein, Zurich and München, under the name of "Bleu coton 4 B," which is said to be identical with "Oxaminblau 4 BX" and "Dianylblau G."

⁴ According to Amann (Ztschr. Wissensch. Mikroskopie, Bd. 13: 18. 1896), lactophenol is composed of: Carbolic acid, chem. pure, crystalline, 20 gr.; lactic acid (spec. grav. 1.31) 20 gr.; glycerine (spec. grav. 1.25) 40 gr.; distilled water 20 ccm.

(II) Bleu coton 4 B	0.02 gr.
Safranin	0.1 gr.
Lactophenol-alcohol (I).....	100 cc.

In the stain (II) the objects are left for two hours, and during this time the glass must occasionally be moved about so as to ensure equal penetration of the dye. After the cuttings have been removed from the stain (II) the lactophenol is washed away with 96 per cent alcohol, and then controlled under the microscope. If the blue staining of the mycelium is not strong enough, the cuttings must be left for a longer time in the solution. If they are too strongly stained the superfluous stain can be removed with lactophenol-alcohol. With this treatment of the cuttings red stains fade more rapidly; hence, when the proper tint of blue has been obtained, the cuttings must be left for about 10–15 minutes in a 0.5 per cent safranin solution in 96 per cent alcohol (III). They are again controlled under the microscope and, after a short rinsing with absolute alcohol, placed in xylol and then in balsam.

Better results are obtained for subsequent staining if, instead of safranin alcohol solution (III), safranin in oil of cloves is used. This process is likewise much simpler and surer. The cuttings are somewhat over-stained in solution (II), the lactophenol is washed out with absolute alcohol, and the cuttings placed in a weak safranin solution in oil of cloves. The cuttings remain in the oil of cloves until the host tissue is stained a deep red (about 20–30 min.). If over-stained, a few drops of pure oil of cloves can be used to neutralize. From the oil of cloves the cuttings are conveyed to xylol and then to balsam.

With proper treatment and differentiation—which are learned after a short experience—the host cells and the mycelium form sharp contrasts of red and blue. The qualities of both stains are easily and surely conveyed to the objects, the bleu coton fairly slowly and the safranin rapidly. Hence a commencement with safranin differentiation must only be made after that with bleu coton has been accomplished. The lactophenol-alcohol allows the cuttings to be conveyed directly from the absolute alcohol to the stain solution, and *vice versa*. Permanent preparations should be over-stained, especially with safranin, as the balsam draws off the stain.

The above process is simple in the case of serial cuttings, and the stain is durable for permanent cuttings. As a rule it is most successful with well-fixed objects (*e.g.*, Juel or Flemming fixatives).

SUMMARY

A method of differential staining is treated which has been worked out in cytological investigations of mycelium of *Plasmopara viticola* in the leaf tissue of *Vitis vinifera*, and can be well and simply applied to other Peronosporaceae.

The microtome cuttings, treated in the usual way with xylol or oil of turpentine to free them from paraffin, are dealt with as follows:

- 1.—10–15 minutes in lactophenol-alcohol (I);
- 2.—Two hours in the stain solution (II);
- 3.—Differentiation under the microscope, the over-stain being eliminated by lactophenol-alcohol;
- 4.—Wash in absolute alcohol;
- 5.—20–30 minutes in a weak safranin solution in oil of cloves;
- 6.—Differentiation under the microscope in pure oil of cloves;
- 7.—Wash with xylol; embed in balsam.

The writer wishes to express his appreciation to Professor Dr. E. Gäumann, under whose direction this work was carried on.

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PYTHIUM ARRHENOMANES N. SP., A PARASITE CAUSING MAIZE ROOT ROT

CHARLES DRECHSLER

Maize rootlets collected in the vicinity of the District of Columbia at various times during several years have yielded an assortment of nearly a dozen different species of *Pythium*. While a number of the isolations represent forms of recognized pathogenicity to a wide variety of cultivated plants, their occurrence in connection with local yellowish or brownish discolorations could not, in the absence of evidence of more serious damage, be regarded as of any considerable pathological moment. More importance presumably attaches to a form submitted for identification by Helen Johann which, in an abstract by Johann, Holbert, and Dickson,¹ was set forth as being associated with root rots of dent corn in Wisconsin and Illinois.

Although the fungus in question, as the abstract states, would seem to resemble the form to which Carpenter² earlier attributed root rot of sugar cane in Hawaii, the resemblance is by no means complete. Against the similarities evident between the two species with respect to the lobulate structure of the zoösporangium, the approximate dimensions of the smooth oogonium and oöspore, and, judging from Carpenter's figures, even the crook-necked shape of the terminally borne antheridium, are opposed rather significant differences in the mycelial relationships of the antheridia and in the number of these bodies to each oogonium. In the account of the cane-root parasite reference is made to "several" antheridia surrounding the female organ, while the illustrations appear to represent from 1 to 5 or at most 6 such structures. In addition it is reported that "Antheridia are often from the same branch as the oogonium." Whereas, in the maize parasite from 15 to 20 antheridia have not infrequently been counted in relation to the upper and equatorial aspects of the oogonium, indicating a probability that the total number, including those concealed underneath, might well lie between 25 and 30. Furthermore, an androgynous condition has never been observed, the mycelial connection between male and female organs being evidently relatively remote as a rule.

¹ JOHANN, H., J. R. HOLBERT and J. G. DICKSON. A *Pythium* seedling blight and root rot of dent corn (Abst.). *Phytopath.* 16: 85. 1926.

² CARPENTER, C. W. Morphological studies of the *Pythium*-like fungi associated with root rot in Hawaii. *Bul. Exp. Sta. Hawaiian Sugar Planters Assoc., Bot. Ser.* 3: 59-65. 1921.

From *Pythium aphanidermatum* (Eds.) Fitz. the maize parasite differs so markedly that the possibility of specific identity is clearly out of question. In the former the almost invariably single antheridium is represented typically by a terminal, subterminal, or intercalary portion of hypha, delimited by 1 or 2 septa, together with a relatively massive orbicular, barrel-shaped or dome-shaped protuberant part making broad apical contact with the oogonium. In the latter the male organ is typically a crock-necked, expanded terminal or lateral structure making narrow contact with the oogonium and never set off from its supporting filament by more than a single cross-wall. It may be noted that inasmuch as in Carpenter's plates the second type of antheridium is illustrated, the conclusion seems unescapable that the identification of the cane parasite as *Pythium aphanidermatum* was quite erroneous.

While the maize root fungus under consideration apparently is not recognizable in any published descriptions based on material from the underground parts of sugar cane, it is not intended to assert at this time that the pathogen may not also occur there. A goodly proportion of several hundred cultures from affected sugar cane roots, obtained in part from greenhouse material collected at Arlington Experiment Farm, Rosslyn, Virginia, but mostly isolated in Louisiana by R. D. Rands from field material, exhibit general similarities to the maize parasite in mycelium and zoösporangial complexes. This is true also of many of the isolations from corn roots to which reference has been made. Final opinion concerning the probable relationship of such forms from the two hosts must await the development of the sexual stage, and that on a substratum permitting satisfactory microscopic study.

Isolations from diseased maize rootlets submitted by W. D. Valleau as typical of the root rot described from Kentucky by Valleau, Karraker, and Johnson³ yielded a preponderance of the "Pythium-like fungus" represented in their illustrations. Its similarity in both sexual and asexual phases is strongly indicative of specific identity with the Wisconsin parasite, which latter may expediently serve as type of a new species for which the term *arrhenomanes*, suggestive of the extraordinary supply of male elements, is proposed.

Pythium arrhenomanes n. sp.

Mycelium intercellular and intracellular; in culture exhibiting moderately strong aerial development; composed of hyphae 2.0–5.5 μ in diameter. Zoösporangia lobulate, composed of inflated communicating elements up to

³ VALLEAU, W. D., P. E. KARRAKER and E. M. JOHNSON. Corn root rot—a soil-borne disease. Jour. Agr. Res. 33: 453–476. 1926.

20 μ or more in diameter, often occurring in extensive compound complexes; evacuation tube usually 3 to 4 μ in diameter, variable in length (frequently 50–75 μ); zoöspores usually from 20 to 50 or more from a vesicle, 2-ciliated, motile, later rounding up as subspherical or ellipsoidal bodies with average diameter of approximately 12 μ , and germinating usually by a single germ tube 2.5 to 3.0 μ in diameter.

Oögonia (on carrot-cornmeal agar) subspherical, terminal or more rarely intercalary, measuring 24–35 μ (average 29.4 μ) in diameter, with a wall approximately 0.5 μ in thickness. Antheridia crook-necked, measuring usually 6–9 μ in diameter in the distal expanded portion, 12–25 μ in length along curved axis from apex to basal septum, the rounded apical end making narrow contact with oogonium about a short fertilization tube that measures approximately 3 μ in diameter, the proximal part more gradually tapering toward delimiting septum to diameter of supporting filament; numerous, from 15 to 20 often visible in relation to an oögonium, the total number then probably in excess of 25; borne terminally or more rarely laterally on branches arising from several (usually 4–8) hyphae, each of which contributes usually up to 4 antheridia, and all of which are distinct from hypha bearing oögonium. Oöspores (on carrot-cornmeal agar) subspherical, yellowish, sometimes completely filling oögonium, usually 22–33 μ (average 27.3 μ) in diameter, containing a reserve globule often 12–19 μ (average 15.4 μ) in diameter, and surrounded by a wall 1.2–2.0 μ (average 1.6 μ) in thickness.

Causing a decay of maize (*Zea mays* L.) roots in Wisconsin.

BUREAU OF PLANT INDUSTRY,
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PHYTOPATHOLOGY

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FLORA W. PATTERSON

1847-1928

BEVERLY T. GALLOWAY

Mrs. Flora W. Patterson, for more than 25 years a member of the staff of the Bureau of Plant Industry, United States Department of Agriculture, died February 5, 1928.

Those of us who measure service in the field of mycology in decades rather than years can look back the somewhat misty pathway and recall our struggles to orient ourselves when mycology began to be pushed into the background by the urgent demand on the part of the public for help in meeting the ravages of numerous crop diseases. There was a period in the early 80's when a mycologist might go his peaceful way collecting and delving in species to his heart's content without fear or distractions of any kind. Mrs. Patterson came to the Department just about the close of this period. The mycologist was passing. The glamor of field service in phytopathology, with its attendant large contacts and large problems, was irresistible so that our collections and herbaria were beginning to languish and our mycological technique becoming rusty for lack of use. To meet the situation, insofar as it related to the Department's work, we tried various expedients and made numerous experiments. It was the conviction of my colleagues of the period that our only hope was to find a good man, rich in experience and so wedded to mycology and its attendant interests that nothing could swerve him from the then recognized beaten path. The experiment was made, but the man failed us.

In our search for relief, we became acquainted with Mrs. Patterson and her work, and on January 15, 1895, she came to us as Assistant Pathologist. Special note should be made of the title, as it is doubtful if we could have succeeded in having her appointed as a mycologist. It soon became evident that Mrs. Patterson's heart and soul were in the work of making our collections something really worth while. This was six or seven years before the organization of the Bureau of Plant Industry and during a period when the groundwork was being laid for the expansion of many lines of botanical work in the Department. In June, 1901, just before the law creating the Bureau of Plant Industry became effective, Mrs. Patterson was made Mycologist, which title she held through more than twenty years of further service in the Department.

It was my privilege and pleasure to be in rather close touch with Mrs. Patterson's work during her long period of service ending in April, 1923. In all that time her loyalty to the collections and all they stood for was unflinching and unswerving. Men and women were coming into the Bureau and moving rapidly forward in the broad field of phytopathology. There was great demand and pressure for workers in this field and the opportunities for advancement were numerous. Mrs. Patterson, so far as I can recall, never became interested in the excitement around her. She had her job and she stuck to it to the end. As the burdens put upon the Bureau became greater, Mrs. Patterson did her share toward lightening them. Thus, when it became necessary to put into operation the Plant Quarantine Law of 1912, Mrs. Patterson and her assistants rendered material aid in organizing and setting in motion the pathological inspection service which has since grown into large proportion.

Mrs. Patterson possessed an active mind and her interests extended much beyond the field of her special work. In the early years of the Bureau of Plant Industry, when policies were being shaped and the groundwork was being laid for an organization in which everyone engaged in research might have freedom of action and the sympathetic support of those engaged in related lines of work, Mrs. Patterson proved a helpful and able adviser. She was cheerful at all times, optimistic in the face of difficulties, and forward looking in all matters pertaining to her work.

Anyone familiar with the steady grind and labor connected with species identification, care of specimens, keeping up with exsiccatae, exchanging plant material, indexing systematic mycological literature, and a multitude of other things associated with a large herbarium in constant use by a score of workers in phytopathology, will realize that little time is left for original research. Despite these handicaps and interruptions, Mrs. Patterson found time to turn out some creditable pieces of research. Her ambition, however, was to serve others by lightening their burdens wherever and whenever possible.

Of the personal side of her life we have little knowledge. We know that she came of good stock, her father being a Methodist minister. We know that for years she had the care of her husband, who, through an accident, was a helpless invalid. Despite these heavy burdens, Mrs. Patterson prepared her two sons for college and managed to enter herself at Radcliffe. She remained at Radcliffe three years, taking special work in botany while working as an assistant in the Gray Herbarium at Harvard. It was while at the Gray Herbarium that she received her special training in mycology and the care of mycological collections. Mrs. Patterson came to the Department direct from the Gray Herbarium, having qualified in 1895 through the usual Civil Service channels. Under the terms of the United

States Retirement Act of 1920, Mrs. Patterson was registered for retirement in 1922. Her period of service, however, was extended for another year, her final retirement taking place April 20, 1923. After retiring, Mrs. Patterson lived quietly with her son. She passed away at the home of her son in Brooklyn, New York. Her friends and co-workers will long remember her as one who could smile through shadow and sunshine, one who was always willing to help, and one whose loyalty to her chosen field remained steadfast to the end.

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PHYTOPHTHORA BLIGHT OF PEONY¹

D. C. COOPER AND C. L. PORTER

The fungus which causes peony blight rather closely resembles *Phytophthora cactorum* and *P. fagi* but has been found to differ from them in morphology, cultural characters, and parasitic capabilities, and is herein designated as *Phytophthora paeoniae*, n. sp. The outstanding morphological characteristics of this fungus are its relatively broad conidia with flat papillae, long twisting antheridial stalks, and more or less spherical haustoria. Only young growing tissues of the peony plant are susceptible to infection, and in the stem the mycelium invades most rapidly the pericycle, medullary rays, and pith.

Oogonia and antheridia are produced in great abundance in pure culture and precede the conidia, the production of which is stimulated by lack of nutrient substances.

Spore production ceased under red and yellow-orange lights. A variety of peculiar morphological and physiological responses were made by the fungus when exposed to the vapors of different volatile substances such as acetone, alcohol, turpentine, oil of bergamot, oil of eucalyptus, clove oil, thymol, menthol, CO₂, and illuminating gas.

Thurston and Orton (12) noted a disease of peony near Erie, Pennsylvania, in 1921, caused by a species of *Phytophthora* which they briefly described. In 1922 a similar disease was reported by White (see Martin, 4, p. 439) as occurring at Shawnee, Kansas. At Hampden, Connecticut, Clinton (see Martin, 5, p. 403) observed the disease and made isolations of the fungus. He obtained both a *Botrytis* and a *Phytophthora* from the blighted stems but considered the *Phytophthora* to be the first invader.

At Lafayette, Indiana, in 1924, Gardner (2, p. 256) observed the disease to be rather serious on the more succulent parts of the plant where it occurred as a stem rot and leaf spot. During the early part of May, 1927, Leslie Pierce, of the U. S. Department of Agriculture, collected a number of blighted stalks in a seven-acre peony farm near Vincennes, Indiana, where the disease was especially prevalent on the varieties Felix Crouse and Festiva maxima. Professor H. H. Whetzel, in a letter to the writers, reported that the blight occurred in New York in 1927. On May 1, 1928, the disease was again observed by Pierce near Vincennes, who said that the peony varieties Felix Crouse, Carl Rosenfeld, and Floral Treasure were

¹ Contribution of the Biological Laboratories, Purdue University, Lafayette, Indiana.

very susceptible to the disease and that the varieties *Festiva maxima* and *Edulis superba* were moderately susceptible. Diseased plants were also obtained from a peony grower near Battle Ground, Indiana.

It is impossible to make an estimate of the total loss caused by the disease. Since it is especially destructive to the growing tips, it will suffice to say that the *Phytophthora* blight is of considerable importance to the cut-flower peony grower.

SYMPTOMS

The *Phytophthora* blight attacks the stems, leaves and buds, the symptoms varying with the age of the plant and the point of attack. When the infection occurs near the tip of a shoot, the blight manifests itself as a necrotic condition of the tip including the leaves and extends for several inches down the stem (Fig. 1, A). The parts affected are dark brown or



FIG. 1.—A. Tip blight caused by the peony *Phytophthora*. B. Early stage of stem infection. C. Later stage just before the collapse of the stalk.

black and somewhat leathery. When the point of infection is farther down the stem, a portion of the stalk several inches in length becomes involved and turns black, and the shoot droops over (Fig. 1, B, C). The central portion of the lesion is black shading toward the edge so that the color of the infected portion gradually merges with the reddish color of the healthy stem. When the young stalks are attacked near the ground line, the entire shoot turns black. Observations by Professor H. S. Jackson indicate that this fungus under field conditions may produce a destructive crown rot.

Under ordinary conditions practically no fungous growth appears on the surface of the diseased portion of the plant. When, however, these parts are placed in a moist chamber or the plants are kept in a saturated atmosphere, an abundant white cottony growth appears on the surface of the invaded parts. Numerous conidia are found on this aerial mycelium. No other spore forms have been found on, or in, the host tissue.

PATHOGENICITY OF THE PEONY FUNGUS

The organism causing the *Phytophthora* blight of peony was isolated May 5, 1927, from material collected by Pierce. The diseased stems were surface sterilized in HgCl_2 , one part to 1,000, for two minutes, then rinsed in sterile water. Transverse sections of the stem about 5 mm. long were made with a flamed scalpel and placed on potato-dextrose-agar plates. Within 48 hours the mycelium had grown away from the sections and transfers were made to agar slants. The fungus was easily isolated without contamination. Usually agar blocks containing mycelium were used for inoculation purposes but, when desired, zoospores were readily obtained by placing conidia in water.

The pathogenicity of the fungus thus isolated was proved by inoculating healthy peony plants. Roots of the *Edulis superba* variety were potted in January, 1928, and grown in the Purdue greenhouse. When the stalks had reached a height of 6 inches, the plants were inoculated by placing pieces of the agar containing mycelium around the uninjured stalks and then placing the plants in moist chambers. After a period of 48 hours the plants were removed from the moist chambers and placed on the greenhouse bench. Six days after inoculation definite lesions had formed at the places of inoculation, and the next day the infected stalks had withered and dropped at the point of the lesion (Fig. 5, A). Checks treated in the same way, except that pieces of agar without the mycelium were used, remained healthy. A second series of plants was similarly inoculated and similar results were obtained. Infection was also readily secured by placing a drop of water containing the zoospores in the axil of the leaf. The fungus was reisolated and compared with the original cultures, with which it was found to agree

in all respects. The controls inoculated with water alone showed no signs of infection. It was found that the disease readily spread to peony plants which were in close proximity to the infected plants when these were watered with a hose.

INOCULATION TESTS WITH OTHER HOSTS

Other plants selected for inoculation with this fungus were bean, tomato, cabbage, sunflower, potato, nasturtium, and broad bean. These were inoculated by placing agar blocks containing the mycelium over a fresh scalpel wound. The inoculated plants were placed in a moist chamber for three days and then removed to the greenhouse bench. No infection occurred on any of these species.

Various roots, fruits, and detached leaves were inoculated to ascertain the host range of this fungus. The specimens to be inoculated were immersed in a weak solution of bichloride of mercury, then rinsed in sterile water and placed in a moist chamber. Inoculations were made by placing a block of agar containing the mycelium on the unbroken surface on one specimen and over an injury made with a flamed scalpel on another specimen. These inoculations were made in duplicate, and a third group of substrata receiving the same treatment but not inoculated was run as a check. All were held at room temperature.

After 10 days in a moist chamber uninjured turnips, parsnips, and carrots showed no infection and only a slight discoloration occurred at the point of inoculation on the surfaces cut with the scalpel. The turnip was blackened below the place of inoculation for a depth of 1-2 mm. and mycelium was found in the blackened tissue.

Ripe tomatoes were attacked readily if the agar block was placed over an injury. The fungus did not gain entrance into the uninjured fruit. Green tomatoes and bell peppers, whether injured or not, were not penetrated by the fungus.

This *Phytophthora* invaded and rotted injured apples but could not enter through the unbroken epidermis. The lesion produced did not have a zonate appearance as does that produced by *P. cactorum*.

Although the fungus was placed on detached living leaves of nasturtium, tomato, *Thalictrum*, hollyhock, soy bean, sunflower, *Nicotiana*, broad bean, and peony, it did not enter the tissue except in the last two mentioned. It caused blackened areas on these leaves and the fungus was easily reisolated from the necrotic spots.

RELATION OF THE FUNGUS TO THE HOST TISSUE

For the purpose of studying the relation of the fungus to the host tissue, portions of the infected peony stalks were fixed in a solution of 6.5 cc.

formaldehyde and 2.5 cc. glacial acetic acid in 100 cc. of 50 per cent alcohol. Permanent mounts were made by the paraffin process. The best stain was found to be Fleming's triple with a very light counterstain in Delafield's haematoxylin. By this method the fungus was very easily differentiated from the host tissue.

A careful study of these slides showed that the fungus advanced from the point of inoculation through the cortex to the pericycle and then encircled the stem through the pericycle in both directions. From the pericycle the fungus passed through the medullary rays to the pith and caused a collapse of the pith cells (Fig. 2, A). Unlike the condition found in the

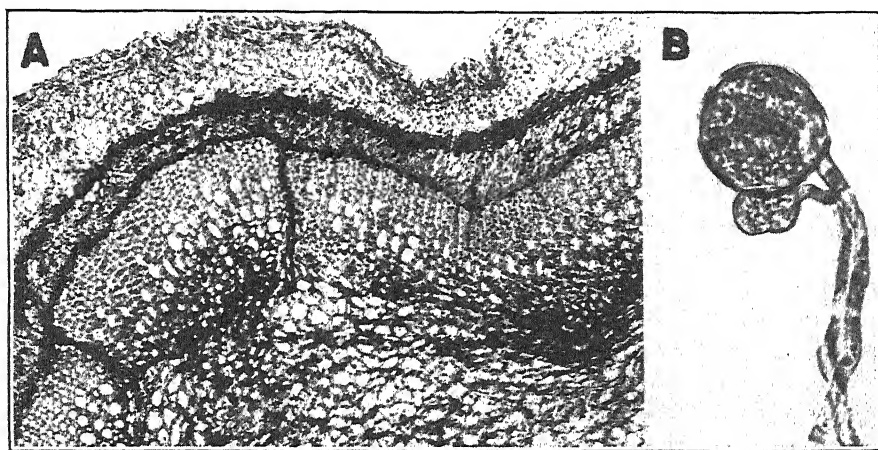


FIG. 2.—A. Cross section of the stem showing pericycle, medullary rays, and pith invaded by the fungus. B. Oogonium and antheridium showing their relatively long stalks and their paragynous relationship.

Phytophthora rots of apple (9), ginseng (10), and strawberry (8), the vascular tissues of the peony were not invaded. The mycelium is intercellular and sends small, more or less spherical haustoria into the cells (Plate XXIX, A). The haustoria have nearly the same diameter as the mycelium but are very much constricted at the point where they pass through the cell walls.

CULTURAL CHARACTERS OF THE PEONY FUNGUS

The peony *Phytophthora* has been grown on a number of the ordinary solid culture media, on cooked vegetable plugs, and in liquid media.

On commercial lima bean agar, growth was very abundant, covering a 90 mm. petri plate in 6–7 days. Both aerial and submerged mycelia were produced. The aerial mycelium, being in scattered tufts, gave the culture

a loose cottony appearance. Unlike certain other species of *Phytophthora* the colony was not zonate in appearance, nor did it have radial strands. The formation of oogonia and antheridia preceded that of conidia, the former being produced on mycelium from 24 to 48 hours old and the latter on mycelium from 4 to 6 days old.

On cornmeal agar the aerial mycelium was less abundant than on lima bean agar. The colony increased slowly in diameter, taking from 10 to 15 days to cover a 90-mm. petri plate. Oospores and conidia were produced in the same sequence as on lima bean agar.

On potato dextrose agar the aerial mycelium was less dense than on cornmeal agar. The colony covered a 90-mm. petri plate in 10–12 days. Oospores and conidia were sparingly produced.

On washed agar the growth was very sparse. No oogonia and antheridia were produced. A few conidia were produced on the older mycelium. On a synthetic medium² and on Bacto nutrient agar the growth was very slight and no oospores or conidia were produced.

On cooked vegetable plugs, after 30 days, the growth was as follows:

Ripe tomato plugs—heavy mat of mycelium, no conidia, many oospores.

Green tomato plugs—heavy mat of mycelium, few conidia, and occasional oospores.

Carrot, parsnip, sweet potato, and green bean plugs—thick mat of mycelium, many oospores, no conidia.

Pepper plugs—thin growth of mycelium, oospores and conidia in abundance.

Green pea plugs and apple plugs—little growth of mycelium, no oospores or conidia.

Peony stem plugs—thick aerial mycelium, oospores in abundance, no conidia, a few chlamydospores. This is the only medium on which chlamydospores were produced.

When the peony *Phytophthora* was grown submerged in beef bouillon, it produced a dense mycelium with no spores. In soil water the thick growth of mycelium produced conidia but no oospores. When grown in maple sap the mycelium produced oospores but no conidia. In prune decoction the mycelium grew luxuriantly, and when it reached the surface of the liquid produced aerial conidiophores and conidia but no oospores.

MORPHOLOGY OF THE PEONY PHYTOPHTHORA

Mycelium. The hyphae are somewhat geniculate and considerably branched. Septa, as a rule, are lacking except for an occasional one in the

² The synthetic agar was made up as follows: distilled water 1,000 cc.; dextrose 100 gms.; peptone 20 gms.; ammonium nitrate 10 gms.; magnesium sulfate (crystals) 2.5 gms.; potassium nitrate 5 gms.; dibasic potassium phosphate 2.5 gms.

older hyphae. The protoplasmic contents are granular and somewhat vacuolate. The hyphae vary from 3 to 10 μ in width. A rapid streaming of the protoplasm in the hyphae may be observed if the culture is examined under the low power of the microscope immediately after the cover of the petri plate is removed. The spore forms will be taken up in the order of their appearance in the colonies grown on lima bean agar.

Sexual organs. For the detailed study of the development of the sexual organs, agar block cultures were used. Slides were prepared by transferring agar blocks from the edge of a petri plate colony to clean slides and placing a cover glass over this agar block. These mounts were kept in moist chambers and examined at intervals.

When permanent mounts were desired, bits of the mycelium were removed from the agar blocks and placed in a drop of 10 per cent formalin for 10 minutes, washed in several changes of water, stained in Congo red and mounted in 10 per cent glycerine. The glycerine was allowed to thicken, a cover glass was put in place, and the mount was sealed with gold size or Duco lacquer. Another method used was to place on a clean slide a drop of lacto-phenol containing 0.5 per cent cotton blue and 0.5 per cent methyl green. In this a bit of the fungus was placed and a cover glass added. With this combination of stains the walls of the oogonium were stained blue and the contents green. These mounts were also made permanent by sealing with gold size or Duco lacquer. When a very thin drop of the mounting medium and a very thin cover glass were used, it was possible to examine these mounts with the oil immersion lens. There was very little distortion of the fungus in either method and the hyphae were beautifully stained.

The oogonia and antheridia arise as terminal swellings of comparatively long, twisting branches from the same or closely adjacent hyphae. (Fig. 1, B; Plate XXIX, G). The antheridium is an ellipsoidal body which is formed somewhat in advance of the thin-walled oogonium. When fully developed the antheridium lies at the base of the oogonium and closely appressed to it. The oogonium when fully developed varies in diameter from 25 to 35 μ . A tube is formed connecting the oogonium and antheridium and through this the contents of the antheridium flow into the oogonium.

The antheridium of this fungus lies at the base of the oogonium, closely appressed to the oogonial stalk. Although occasionally, upon superficial examination, the antheridium appears to have an amphigynous relation to the oogonium such as was described by Murphy (6), yet by careful focusing of the microscope, and by a study of the mode of development of these organs, no case was observed where the oogonium actually grew through

the antheridium (amphigyny) (Plate XXIX, C, D, E). The antheridium is very clearly at the side of the oogonial stalk when observed before fertilization occurs. After the antheridial contents are transferred to the oogonium through the fertilization tube, the antheridium is a flaccid hyaline empty sac which sometimes appears to collapse around the oogonial stalk (Plate XXIX, D).

With the completion of fertilization a thick-walled oospore is formed. These are abundant in older cultures on most agars and vegetable plugs and in soil water. The oospores are globose, with thick walls and a vacuolate central portion, and vary from 24 to 30 μ in diameter, the mode being 26 μ . Oospores from a one-month-old colony germinated by production of a germ tube if they were placed in a weak sugar solution. As previously stated, no oospores have been found either on or in the diseased peony tissue.

Conidia. The conidiophores are formed on the older parts of the colony but have not been found on mycelium less than 4 or 5 days old. They are prostrate on the surface of the medium. Their diameter is slightly less than that of the mycelium from which they arise. Each conidiophore bears from 1 to 15 conidia on short lateral branches, the usual number being from 4 to 8. The conidia are ovoid with broad apical papillae and are pale yellowish gray in color with granular and often vacuolate contents (Plate XXIX, B). They vary in size from 22 to 38 μ by 26 to 42 μ , mostly from 26 to 28 μ by 30 to 33 μ .

Zoospores. The conidia usually germinate by the formation of zoospores. When the conidia are placed in a drop of water the contents surrounding the large central vacuole break up into 20–40 individual units which round up into zoospores (Plate XXIX, B). In a short time these spores exhibit slight movement due to the rapid vibration of the cilia. With the increase in size and activity of the zoospores the central vacuole of the conidium entirely disappears.

The zoospores escape through the apical plug which is ruptured because of the internal movement. Usually this escape is very rapid. Sometimes a few of the zoospores escape and relieve the internal pressure. The remaining spores become very active, each one escaping from the conidium by squeezing through the apical opening or orifice, which has a smaller diameter than the zoospore itself. In the first stage of its escape, the anterior end of the zoospore is squeezed into the orifice. The posterior end then seems to contract, forcing the contents of the spore forward. The protruding anterior end becomes swollen beyond the orifice so that the partially extruded zoospore assumes the shape of an hour-glass. By further contraction the entire contents continue to flow forward, and the spore

aided by its cilia escapes with a spiral movement. In some cases a few of the zoospores do not escape but come to rest and germinate within the conidium (Plate XXIX, B3). When zoospores are not formed within a period of a few hours, the conidium produces germ tubes directly, usually through the apex, sometimes laterally (Plate XXIX, B2).

The zoospores are reniform in shape with two cilia attached near the middle of the concave side. After a short period of brisk activity, the spores gradually come to rest and assume a spherical shape, losing or absorbing the cilia. Almost immediately germ tubes are sent out so that in the space of an hour after the production of zoospores, hyphae are being developed (Plate XXIX, B4).

Chlamydospores. The chlamydospores are spherical and thin-walled with granular contents. They have been observed only on cooked peony stems and then were relatively few in number. The majority of these spores range in diameter from 30 to 40 μ . Germination of the chlamydospores has not been observed.

EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF THE FUNGUS IN CULTURE

Tests were made to determine the optimum temperature for the growth of the fungus. Lima bean agar was used because it was known that the fungus grows best on this medium. Petri plates for each temperature were prepared in duplicate and the entire test repeated to check results. Temperatures of 14° and 18° C. were obtained in a refrigerator and temperatures of 22°, 26°, 30°, 34°, and 37° C. in incubators under thermostatic control. All the cultures in the incubators were kept in moist chambers to prevent the medium from drying. The plates were inoculated and the colonies allowed to grow for one day and then measured before the plates were placed in the constant temperature chambers.

The daily increase in the diameter of the colonies was recorded at 4 p. m. each day for a period of 7 days. The average daily increase at the various temperatures was 3 mm. at 14° C., 7 mm. at 18° C., 14 mm. at 22° and at 26° C., 12 mm. at 30° C., 1 mm. at 34° C., and 0 mm. at 37° C. From these measurements it can be seen that the optimum temperature for the growth of this fungus in culture lies between 22° and 26° C., with a sharp break above 30° C. In every case the fungus was dead when left at 37° C. for a period of 7 days.

Tisdale and Kelley (13) found the optimum temperature for the growth of *Phytophthora nicotianae* to be between 25° and 30° C. Rose (8) considers the best temperature for the growth of *P. cactorum* to be between 25° and 36° C. At room temperature cultures of the peony *Phytophthora*

may be held for periods of two to three months without loss of vitality. Church and Scandiffo (1) also found room temperature best for the storage of *Phytophthora* cultures.

EFFECT OF THE H-ION CONCENTRATION OF CULTURE MEDIA
ON THE GROWTH OF THE FUNGUS

Lima bean agar was made up and, when tested, was found to have a pH of 6.6. This was divided into three parts, one part was used as it was, another was adjusted to a pH of 5.3 by adding lactic acid, and the third part adjusted to a pH of 8.4 by the addition of NaOH. Petri plates of each of the media were prepared in triplicate and after inoculation were held at room temperature for 5 days. An effort was made in all cases to use the same amount of inoculum.

At the end of the test it was found that the colonies on the media having a pH of 5.3 had an average diameter of 32 mm., those on media having a pH of 6.6 had an average diameter of 61 mm. and those on media having a pH of 8.4 had an average diameter of 41 mm. In so far as vegetative growth was concerned, the most favorable H-ion concentration for the fungus was the medium having a pH of 6.6. A microscopic examination revealed the fact that the colonies at a pH of 6.6 and 8.4 bore numerous oospores, whereas the colony growing on agar having a pH of 5.3 bore comparatively few conidia or oospores. Tisdale and Kelley (13), in their work on *Phytophthora nicotianae*, found the H-ion concentration most favorable for vegetative growth of the fungus to be between a pH of 4.4 and pH 5.1.

When brom-cresol purple was added to neutral beef bouillon as an indicator, and the fungus grown submerged in the liquid, there was no color change even though the culture was kept for six weeks, thus indicating no change in the pH.

THE EFFECT OF CERTAIN VARIATIONS OF RICHARD'S SOLUTION ON
THE GROWTH OF THE PEONY PHYTOPHTHORA

The peony *Phytophthora* grew well in Richard's solution, producing a thick mat of submerged mycelium but no spores. If the solution was varied by adding either lactose or saccharose for the glucose, there seemed to be no difference in the amount or type of mycelium produced. Even when albumen was substituted for the KNO_3 there was no visible variation in the fungous growth. When urea salts were substituted for the KNO_3 there was a more abundant growth of mycelium, the hyphae were peculiarly twisted, and many conidia were produced.

When either CuSO_4 , HgCl_2 , ZnSO_4 , tartaric acid, or phenol were added to Richard's solution at the rate of 1 gr. to 1,000 cc. there was no growth of the fungus.

EFFECT OF VARIOUS VOLATILE SUBSTANCES ON THE VEGETATIVE
GROWTH OF THE FUNGUS

Cultures of the peony *Phytophthora* were grown on lima bean agar in petri plates until a diameter of 25–30 cc. was reached. The petri dish cultures were then placed in culture-dish chambers and a small piece of cotton placed at the side of the petri dish. On the cotton were placed a few drops (not to exceed 3 cc.) of a volatile substance, the lid was removed from the petri plate, and the culture-dish chamber was then covered. After a period of 60 hours at room temperature, the increase in the diameter of the colony was noted and the colony was also examined under the low power of the microscope to determine the effect of the substance on the vegetative growth. The lids were then placed on the petri dishes and the cultures allowed to stand 48 hours longer to determine whether the fungus was still alive. These experiments were carried on in such a way as to guard against contamination as much as possible, and very little contamination occurred.

The hyphae which were exposed to the vapors of acetone and of 25 per cent alcohol were much curled (Fig. 3). When oil of bergamot and menthol were used, there were many short, club-shaped branches of the mycelium. Turpentine caused the aerial hyphae to curl, and the submerged hyphae were club-shaped, many bursting at the end. Oil of eucalyptus caused rounded, knob-like swellings on the hyphae. Thymol caused a profuse branching of the hyphae at their tips. Clove oil caused the hyphae to be sinuous. The fungus was found to be dead in all cases except when thymol and clove oil were used.

When the colonies were kept in a chamber containing illuminating gas, the mycelium developed many warty protrusions and was finally killed. In CO₂ the fungus was profusely branched, but after being removed from the gas continued to grow. However, during the 4 days the culture was kept it failed to produce oospores, but did produce conidia in abundance. This was of interest since the fungus usually produces oogonia and antheridia upon the new growth.

THE EFFECT OF CERTAIN COLORS OF LIGHT ON THE DEVELOPMENT
OF THE FUNGUS

Cultures of the fungus two days old were placed in light of different wave lengths obtained by the use of ray filters and left there for four days. The ray filters used were as follows: red, transmitting red only; yellow orange, having a high transmission of red and green with a sharp cut off beyond blue green; amber, absorbing the blue and ultra-violet; blue, transmitting approximately primary blue; bluish tint, transmitting a full spec-

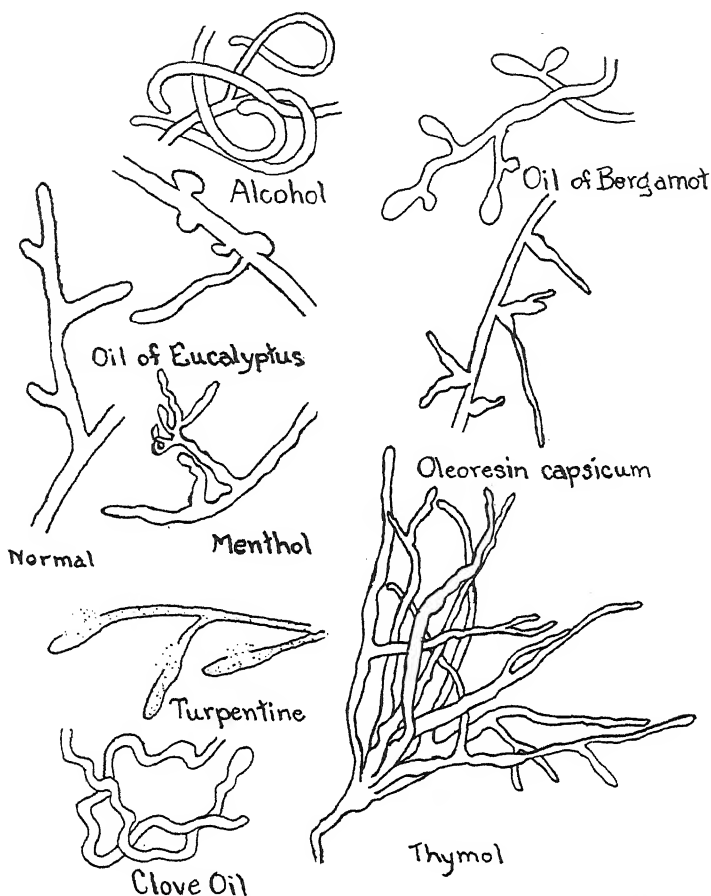


FIG. 3. Effect of certain volatile substances on the vegetative growth of the fungus.

trum but absorbing the infra red; smoky violet, completely removing the yellows; and canary, transmitting the full spectrum. The vegetative growth was about the same in all cases. When the red and yellow-orange filters were used, no conidia or oospores were produced; whereas they were produced in abundance under all the other filters tested.

THE PHENOMENON OF STALING AND INHIBITION

There seemed to be no definite staling effect of the colony of the peony *Phytophthora* upon the surrounding medium since the colony appeared to grow without any apparent diminishing rate. Leonian (3) found no staling phenomena in the *Phytophthora* species he studied. When two colonies of the peony *Phytophthora* were grown upon opposite sides of a petri plate,

the colonies grew together, the hyphal strands intermingled, and there was seemingly no inhibition. However, if *P. erythroseptica* was grown on one side of the plate and the peony *Phytophthora* on the other, there seemed to be a distinct antagonism. The hyphae did not intermingle and a strip of agar 1-2 mm. wide remained clear between the colonies. Along either side of this strip the fungi produced conidiophores instead of the usual oogonia and antheridia (Fig. 4). This agrees with the results obtained by Porter (7) when working with other fungi.

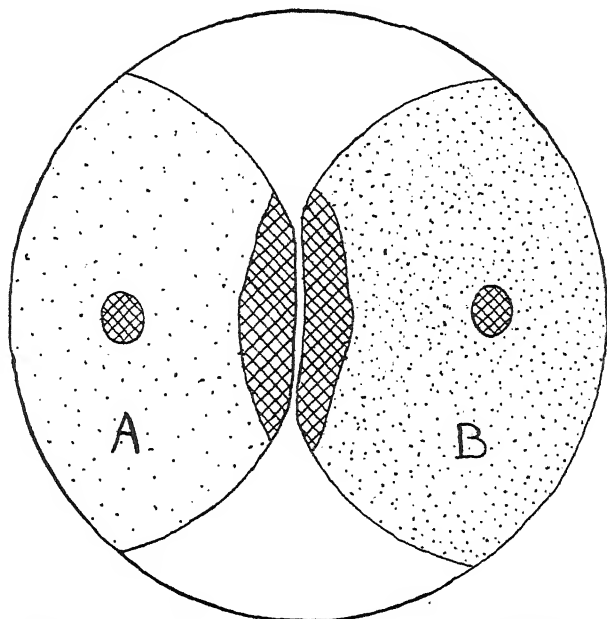


FIG. 4. Diagrammatic drawing of the inhibition exhibited between a colony of *P. erythroseptica* and the peony *Phytophthora*. The cross-hatched areas show the portions of the colonies in which conidia were formed. A. *Phytophthora* from peony. B. *Phytophthora erythroseptica*.

Since conidia are produced in abundance on the older portions of the colony, on the advancing mycelium when the surrounding agar is cut away, on the advancing mycelium when two colonies of different species approach each other, and when the fungus is grown on washed agar, it would seem that the production of conidia is stimulated by the absence of nutrient material.

COMPARISON OF THE PEONY PHYTOPHTHORA WITH OTHER PHYTOPHTHORA SPECIES

After the study of the peony fungus was begun, a number of the described species of *Phytophthora* were brought together for comparison.

Cultures of the following species were procured:

P. parasitica Dastur was obtained from J. B. Kendrick, Purdue University; *P. terrestris* Sherb., *P. colocasiae* Rac., *P. cryptogea* Pethyb. and Laf., *P. fagi* (Hartig) Hartig, *P. pipi* Leonian, *P. mexicana* Hotson and Hartge, and *P. palmivora* (Butl.) Butl. were obtained from Leon H. Leonian, Morgantown, W. Va.; *P. erythroseptica* Pethyb. was obtained from G. H. Pethybridge, England, and from the Centraalbureau, Baarn, Holland; *P. richardiae* Buisman was also obtained from Holland; a *Phytophthora* causing stump root rot of lily and another isolated from cotton boll were obtained from C. M. Tucker, Porto Rico; a *Phytophthora* from *Hevea*, one from abaca, and Reddick's tomato *Phytophthora* were obtained from the Department of Plant Pathology, University of Wisconsin; *P. infestans* (Mont.) DeBary was obtained from R. W. Samson, Purdue University, and *P. cactorum* (Lebert and Cohn) Schröt. was obtained from M. W. Gardner, Purdue University, and was also isolated from apples by the writers. Because of the very different type of their mycelial growth on lima bean agar, all of these except *P. cactorum*, *P. fagi*, and *P. erythroseptica* were distinctly different from the peony fungus.

On all the solid culture media, the peony *Phytophthora* grew better than did *P. cactorum*. As compared with the loose, cottony tufted appearance of the cultures of the peony *Phytophthora* on lima bean agar, the culture of *P. cactorum* had a smooth, thick, velvety appearance.

When the growth of the peony *Phytophthora* on various vegetable plugs was compared with that of *P. cactorum* on similar plugs, interesting cultural differences were observed. While the peony *Phytophthora* grown on sterilized peony stems produced a thick aerial growth with numerous oospores and a few chlamydospores, *P. cactorum* on a similar substratum produced a very scanty mycelium with only a few oospores. On the other hand, on apple tissue and on green bean pods, *P. cactorum* produced numerous hyphae and abundant oospores as compared with the slight aerial growth produced by the peony *Phytophthora* and its complete absence of spores. On the tomato plugs the mycelial growth of the two fungi was similar except that the peony *Phytophthora* produced oospores and conidia to a much greater extent than did *P. cactorum*. In order to check these results, the work was repeated, and a series of cultures of *P. erythroseptica* was used for comparison. It was found that *P. erythroseptica* was more like the peony *Phytophthora* in the amount of mycelial growth and spore production than was *P. cactorum*.

It is of interest to note that *P. cactorum* caused a complete rotting of both green and ripe tomatoes when the inoculum was placed over an injury. *Phytophthora parasitica* was able to penetrate the uninjured fruit

whether green or ripe, whereas the peony *Phytophthora* attacked only the ripe fruit and then only through an injury.

The peony fungus caused injured Grimes apples to decay but did not enter through the unbroken epidermis, whereas *P. cactorum* readily penetrated through the uninjured surface. The lesion on the apple caused by the peony *Phytophthora* did not have the zonate appearance characteristic of the lesions caused by *P. cactorum*. Upon examination of the apple tissue infected with *P. cactorum*, it was found that the fungus sends into the host cells long, finger-like haustoria similar to those described by Rosenbaum (11). In the apple tissue invaded by the peony *Phytophthora* the haustoria were similar to those which this fungus produced on the peony tissue (more or less spherical) but distinctly different from the haustoria of *P. cactorum*.

Inoculation of the peony was attempted by using *P. erythroseptica*, *P. parasitica*, *P. richardiae*, *P. fagi*, *P. cactorum*, *P. terrestris* and the *Phytophthora* causing stump root rot of lily. In no case did the fungus enter through the unwounded tissue. When inoculation was again attempted by injuring the surface on which the inoculum was placed, *P. cactorum* was the only one of these species that entered the plant and it caused merely a small lesion on the side of the stalk (Fig. 5, B).

All of the described species of the genus *Phytophthora* have many characters in common, so that it is necessary to consider each of them before attempting to determine the taxonomic position of the peony *Phytophthora*. At the start such species as *Phytophthora omnivora* DeBary, *P. erythroseptica* Pethyb., *P. infestans* (Mont.) DeBary, *P. phaseoli* Thaxt., *P. parasitica* Dastur, and others having a so-called amphigynous relationship of the sexual organs, can be set aside. *Phytophthora jatrophae* Jensen, *P. faberi* Mont., and *P. nicotianae* Breda de Haan are also eliminated because of their dense aerial growth and the absence of sexual organs on lima bean agar. This would leave among the described species *P. fagi* (Hartig) Hartig, *P. syringae* (Klebh.) Klebh., and *P. cactorum* (Lebert and Cohn) Schrot, as being more or less similar to the peony *Phytophthora*.

Phytophthora fagi differs from the peony fungus in that it produces on lima bean agar a uniformly dense white aerial mycelium. The conidia are more elongated and have a constant difference of 8-10 μ between the length and width and also have much more prominent apical papillae.

P. syringae differs distinctly from the peony *Phytophthora* in that the conidia are described as having various shapes, the length sometimes being twice the width. According to Rosenbaum (11) the submerged hyphae are much twisted and gnarled on potato agar and there is an absence of spore forms on lima bean agar. The peony *Phytophthora* on potato agar produced spores and normal mycelium.

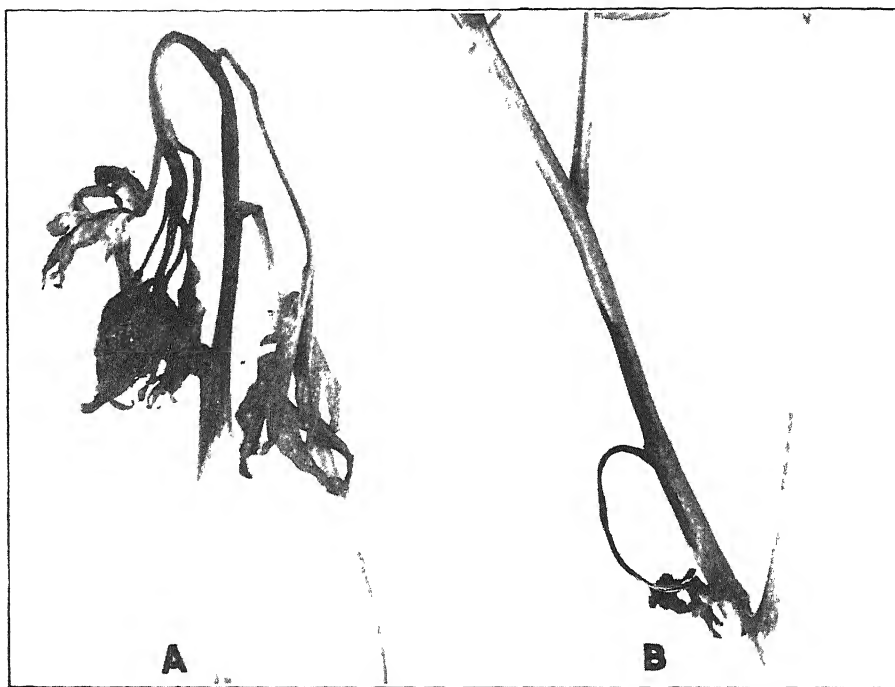


FIG. 5. A. Tip blight of peony caused by the peony *Phytophthora* one week after inoculation. B. Lesion on a peony stem produced by *P. cactorum* after three weeks.

The morphological differences are less marked between the peony fungus and *P. cactorum*. The long twining stalks of the oogonia and antheridia that are found in the peony *Phytophthora* are not found in *P. cactorum*. The apical papillae of the conidia of *P. cactorum* are prominent, whereas those of the peony fungus are broad and more or less flattened. The difference between the average lengths and widths of the conidia of *P. cactorum* is 9–11 μ , whereas the average difference for the peony *Phytophthora* is 4–6 μ . In culture the aerial mycelium of *P. cactorum* is uniformly dense while that of the peony fungus is in scattered, loose, cottony tufts. In its host tissue the peony *Phytophthora* produces small, more or less spherical haustoria instead of the long finger-like projections of *P. cactorum*. All attempts to inoculate peonies with *P. cactorum* have failed to cause the typical *Phytophthora* blight.

These facts indicate that the peony *Phytophthora* apparently is an undescribed species, and the name *Phytophthora paeoniae* n. sp. is proposed.

Phytophthora paeoniae n. sp.

Mycelium at first continuous and granular, somewhat geniculate, later becoming vacuolate and finally sparingly septate, 7–10 μ in diameter, intercellular, producing small sub-spherical haustoria; conidiophores not distinguishable from the mycelium; produced on mycelium of colony from 4 to 5 days old; bear from 1 to 15 conidia; conidia terminal on short unbranched lateral hyphae, normally ovate and papillate, the papillae being usually broad and flat; conidia measure 22.5–35.6 μ by 26.2–41.2 μ , mostly 26–28 μ by 31–33 μ , germinating by zoospores, sometimes by germ tube; zoospores biciliate, 8–10 μ by 10–12 μ , becoming globose, and 8–10 μ in diameter; chlamydospores few, 30–40 μ in diameter; oogonia in abundance on mycelium from 24 to 48 hours old and older, 26–34 μ in diameter, pale to brownish in color; antheridium oval to globose, closely appressed to base of oogonium at side of oogonial stalk; oospores globose, thick walled, 24–30 μ in diameter; formed profusely on most culture media.

Parasitic on the tips, stalks, and leaves of peony in Indiana, Pennsylvania, Connecticut, and Kansas, United States of America.

SUMMARY

1. The *Phytophthora* blight of peony is primarily a tip and stem blight, but often appears as a leaf spot. The infected portions turn black and wither.

2. Inoculation experiments with pure cultures show that the peony stalks are readily blighted by this fungus. Reisolations were made. Inoculations were attempted with eight other species of *Phytophthora* but without success.

3. The mycelium is intercellular with more or less spherical haustoria extending into the cells of the host tissue. It was found in the cortex, pericycle, medullary rays, and pith. No spores were found in the host tissue.

4. The oogonia and antheridia are produced on long hyphae, the antheridial stalk often twisting about the oogonial stalk.

5. The conidia are much shorter and broader than those usually found in the genus *Phytophthora*.

6. Chlamydospores have been found only in cultures on sterilized peony stems.

7. The optimum temperature for the growth of the fungus is between 20° and 26° C. The minimum is about 14° C. and the maximum about 34° C.

8. Peculiar morphological reactions to the vapors of various volatile substances tested were observed in cultures of this fungus.

9. Spores were produced in all lights tested except under red and yellow-orange filters.

10. The fungus grew best on a culture medium having a pH of about 6.6. It grew better on a medium having a pH of 8.4 than on a medium with a pH of 5.2.

11. Growth of the fungus was not inhibited by its staling products.

12. Although a colony of the peony *Phytophthora* was not inhibited by another of the same species, yet there was marked inhibition between this fungus and *P. erythroseptica*.

13. The peony *Phytophthora* differs from previously described species, and particularly from *P. cactorum* which it most nearly resembles, in cultural characters, shape of conidia and haustoria, length and twining character of the antheridial stalk, and pathogenicity. It has been described herein as *Phytophthora paeoniae* n. sp.

The writers feel deeply indebted to Dr. M. W. Gardner, of the Agricultural Experiment Station, for his generous assistance during the course of this investigation and to Professor E. J. Kohl, who made the photographs and photomicrographs.

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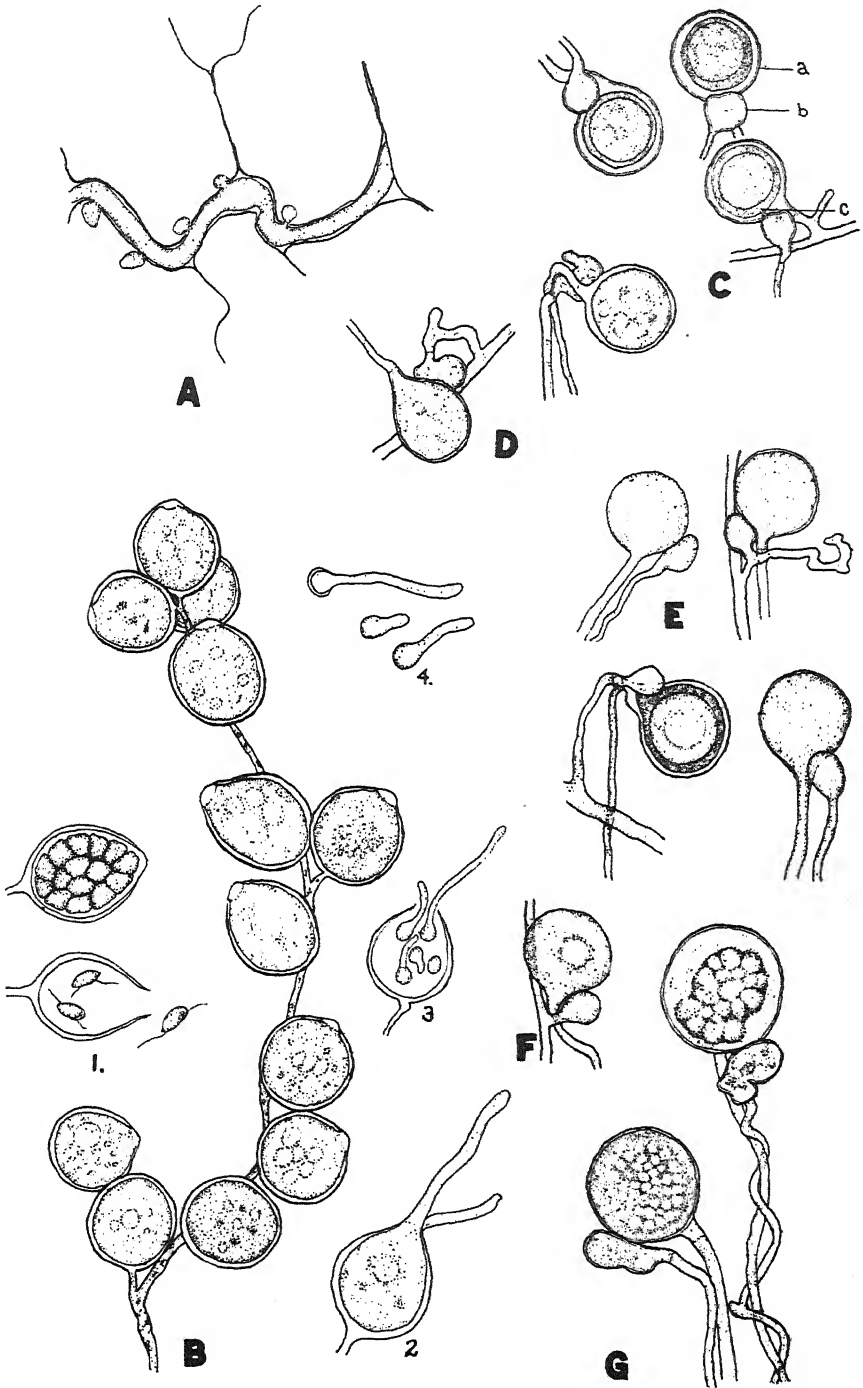
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EXPLANATION OF PLATE XXIX

Camera lucida drawings of *Phytophthora paeoniae* n. sp. ($\times 400$)

- A. Intercellular mycelium in peony stem with small, more or less spherical haustoria extending into the cells of the host tissue.
- B. Typical conidiophore bearing conidia. 1. Conidia germinating by production of swarm spores. 2. Conidium germinating by production of germ tubes. 3 and 4. Germinating zoospores.
- C. Three mature oospores showing relation of oogonium (a) to antheridium (b). Note fertilization tube (c).
- D. Two oogonia and antheridia before fertilization. The figure at the right shows how the antheridium sometimes has a tendency to surround the oogonial stalk.
- E. Four oogonia and antheridia in early stages of development.
- F. Early stage showing antheridium closely appressed to base of oogonium.
- G. Oogonia and antheridia showing their relatively long supporting hyphae. The right-hand group shows how the antheridial stalk oftentimes entwines around the oogonial stalk.



LOCALIZATION OF RESISTANCE TO POWDERY MILDEW IN THE BARLEY PLANT

J. R. MACKIE

The investigations described in this paper represent an endeavor to delimit more definitely the features of varietal resistance of barleys to the attacks of powdery mildew, and an inquiry into the likely causes of this resistance with the view of obtaining some light on the probability of breeding or selecting a barley permanently immune to the attacks of *Erysiphe graminis* D.C.

The author undertook some morphological and cultural studies of the fungus collected in several localities in California, but could find no noticeable differences from the mildew previously described on barley. However, the severe damage caused by mildew during the last few years could not be overlooked. The loss sometimes included the whole grain crop, the fields having to be cut for feed.

THE DISEASE

The symptoms of the attack are marked. The first sign is the appearance of white flecks of mycelium upon the leaves, which increase rapidly in size and are covered with powdery conidia in a few days. There follows a gradual but complete yellowing of the whole plant with the exception of the areas immediately surrounding the infected epidermal cells. These spots remain green for a considerable period after the yellowing of the rest of the leaf.

A cessation of growth results at a time (usually in March and April) which is normally the period of greatest vegetative development for the plant, *i.e.*, the tillering period. The yellowing of the entire plant points suggestively to the secretion of a substance of toxic nature by the fungus. Soon, however, the plant sends out new leaves, and these, appearing at a time when the drier and brighter weather tends to suppress the mildew, manage to retain their vitality, although scattered spots of infection develop. Notwithstanding this new growth, the plant never regains its original vigor, but is smaller than normal, with fewer tillers, since many die before heading. Maturity is delayed. The heads that mature contain many abortive kernels at both basal and apical ends, those kernels that develop being shriveled and poorly filled. Toward the end of ripening there is a darkening in the region of the germ. Since the fungus does not attack the head, these effects are indirect.

The injuries mentioned above destroy the export value of the crop and put it in the feed, or low price, class.

THE MILDEW FUNGUS AS OBSERVED ON BARLEY

In the Erysiphaceae the germinating conidium forms a flattened appressorium against the wall of a cell of the appropriate host (usually an epidermal cell), and from this a slender infection thread grows down into the cell. A reaction, supposedly enzymatic, takes place around the point of this infection thread making a circular depressed area which in many cases remains colorless in stained preparations (Figs. 1 and 3). An outgrowth

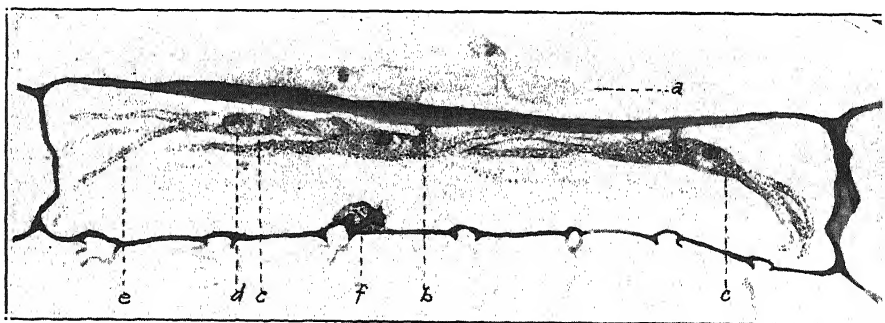


FIG. 1. Eight-day culture of mildew on Atlas barley. Showing superficial mycelium (a), and infection thread (b), several large haustoria (c) with prominent nuclei (d) and lobed ends (e). The host nucleus (f) is not involved. ($\times 400$)

or swelling of the inner layer of the host cell wall accompanies the penetration of the thread, and after penetration surrounds it like a collar, frequently becoming a granular or disintegrated layer about the haustorium. In *Erysiphe graminis* the haustorium is branched at one end, or more characteristically at both ends, into several finger-like lobes. There is a single central nucleus in the main body of the haustorium. (Fig. 3.) Mycelium development in this species is external and chains of conidia are produced on erect branches from the mycelium, while the perithecia are formed partially imbedded in it. Some genera and species of the Erysiphaceae, however, are endophytic.

The assumption of early mycologists that morphological identity delineates a species has been modified by the discovery that within a single morphological species of a parasitic fungus there may be several physiological strains, or forms, which usually can be distinguished only by their cultural behavior.

In the Erysiphaceae the specialization is found both in ascospores and conidia (8).

SUSCEPTIBILITY AND RESISTANCE TO MILDEW

Resistance and susceptibility are variable qualities. The susceptibility of a host to a given physiological form of infecting organism has been noted to vary with change of locality, and to be different in different years and seasons. Since this is the case, physiological specialization can be said to hold strictly only under a definite set of conditions with a sufficiently large number of trials to assure uniformity.

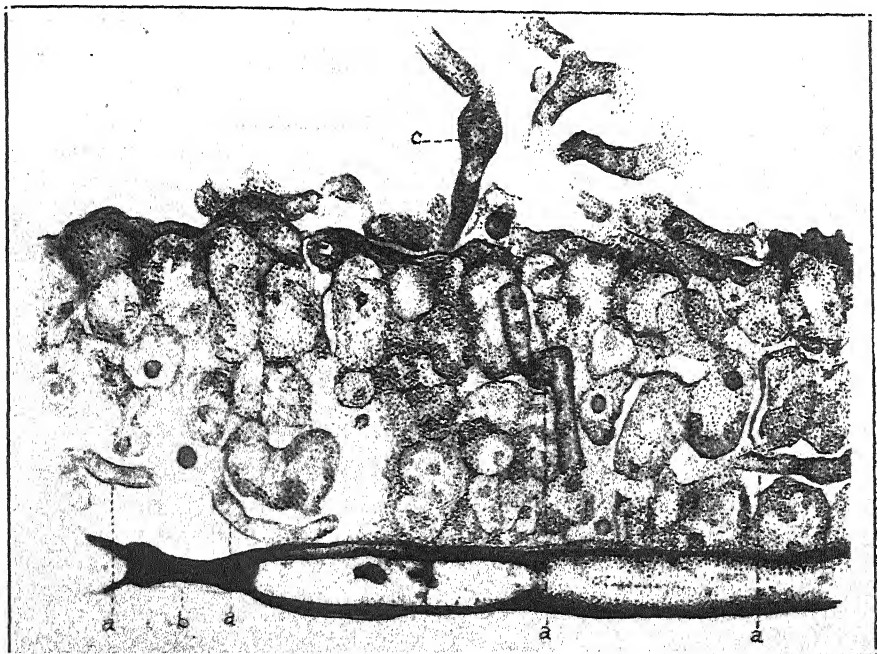


FIG. 2. Eight-day culture of mildew on exposed mesophyll of ventral surface on Hero barley. Showing intercellular hyphae (a), stoma of the host (b), and conidiophore (c) growing from scraped surface. ($\times 400$)

All gradations seem to exist between true susceptibility and absolute resistance, and at least three types of reaction between mildew and its hosts have been observed. Neger (5) showed that on resistant hosts the conidia of the mildew germinate, but soon die without having caused penetration of the host tissue. On other host plants haustoria are produced, but the host cells become filled with gum, stopping the growth of the parasite. If occasionally a haustorium escapes and continues to grow, this constitutes what Salmon, according to Neger (5), terms "subinfection." Salmon (12) found that the disintegration of the haustoria of *Erysiphe graminis* occurred in from one to six days when they developed in unsuitable hosts.

The inoculated plants were kept for four days under a bell jar, then placed under the cage. Abundant conidia formed on these plants and were used as the stock source for the following experiments.

FIELD TESTS

1. Early in February, 1927, Atlas, Sacramento, Hero, and Chile were sown in double rows in the field, and after the appearance of the fourth leaf sprayed with sterile distilled water and then with a fresh suspension of ripe conidia. These plants were not covered. Both Atlas and Hero developed mildew patches by the fourth day, conidia appearing in six days. No mildew developed on Sacramento, but after a week several small patches were observed on scattered plants of Chile. The check rows were not sprayed with the inoculum. Scattered spots of infection appeared on the checks of Hero and Atlas after five days, but none on the Sacramento or Chile. Sacramento being slow growing, another inoculation was made on it two weeks later, but no infection occurred.

2. Later, in March, all eight varieties were planted in 8-inch pots, three plants to the pot. They were germinated in the greenhouse until the hypocotyl was visible, then removed to the field and sunk to the level of the soil under close cheesecloth cages, four pots to the cage. These plants were watered carefully so that no water (containing substances possibly toxic to mildew) splashed upon the leaves. After the appearance of the fourth leaf on all varieties, the leaves were sprayed with sterile distilled water, and three leaves on each plant were inoculated by means of a scalpel sterilized in alcohol with conidia from the stock plants. (A pot of each variety was kept as check under a separate cage, but no mildew developed on any of these checks until after the removal of the cage.) The conidia were applied $1\frac{1}{2}$ inches above the top of the leaf sheath in each case and the leaf marked by a bit of thread tied loosely about the base. The plants were allowed to grow for ten days under the cages. The cheesecloth was then removed on three sides to reduce the temperature. Examinations of the inoculated leaves were made frequently, and the following tentative rating given the varieties: Atlas 4°, Sacramento 0°, Hero 3°-4°, Chile 1°-2°, Gold-foil 0°, Hanna 4°, Oderbrucker 2°-4°, and Common Chile 0°.

3. Damp chamber experiments were carried out with all eight varieties under the same conditions of germination, age, washing, etc., as above. Single conidium inoculations were made on the leaves of plants grown in 6-inch pots, two plants to a pot. Three leaves were inoculated on one plant in each pot, the other plant being kept as a check. In no case did the latter become infected until after the removal of the jars. The inoculations were made with a fine glass rod which was touched to the spores of the stock culture, examined under a low power objective to be sure of one conidium, and

the conidium placed $1\frac{1}{2}$ inches above the top of the sheath on moist, marked leaves. The plants were kept under a bell jar for five days, ventilation being secured through cotton placed between the slightly raised edge of the bell jar and the soil of the pot. At the end of this period the bell jars were removed and a careful examination was made of the inoculated leaves. What seemed to be subinfection occurred on both Chile and Common Chile. No infection appeared on Sacramento or Goldfoil. All the others developed a moderately heavy growth of conidia which increased rapidly after the removal of the bell jars. True infection appeared on the Chile plants in three cases after the removal of the jars.

4. Petri dish tests were made with all eight varieties. Two series were run for each variety, one containing two leaves placed ventral surface down, and the other containing two leaves ventral surface uppermost. The petri dishes had been sterilized in a steam autoclave for one-half hour at 15 lbs. after four layers of paper toweling and considerable water had been placed in them. The leaves, while on the plants, were washed in sterile distilled water and portions of two from each plant were cut off near the center of the blade and placed with sterile forceps in a petri dish with the corresponding surface uppermost in both. In each case one of the pieces of leaf was inoculated by a sterile scalpel with several conidia, the other piece being left as a check. In no case did the check show mildew infection, although in two cases saprophytic fungi started to grow at the cut edges after about five days. The dishes were placed where they got direct morning sunlight and indirect afternoon light. After four days brownish spots had appeared on the dorsal surface series of Common Chile beneath the spot of inoculation, but no other indications of growth were apparent. Mycelia appeared on the dorsal surface series of Chile, but not on the ventral. No infection was obtained on Sacramento or Goldfoil. True infection with conidial formation appeared on the inoculated spots on both surfaces of Atlas, Hero, Hanna, and Oderbrucker. As a result of these studies the rating of the varieties was considered to be: Atlas 4°, Sacramento 0°, Hero 3°-4°, Chile 1°-3°, Goldfoil 0°, Hanna 4°, Oderbrucker 2°-4°, and Common Chile 0°-1°.

MICROSCOPICAL STUDIES

1. A series of tests (series 1-7) was run to determine the best conditions for fixing, sectioning, staining, etc. Atlas and Hero were used for these preliminary tests and the leaves were taken from three to nine days after inoculation. Two formulae for fixing solutions were tried, the material being kept in these for three days at room temperature, 33° and 32° F. Uninoculated leaves were also run under the same conditions. One fixative

used was the Wisconsin¹ (one-half strength). This seemed to hold the shape of the host cells better than the second fixative, but the sections were more difficult to stain. The second fixative² seemed the more satisfactory. Sections 12 μ in thickness were found to be best, although they were good down to 10 μ . Various stains were tried, but the triple stain (safranin, gentian violet, and orange G) gave the best results. On the whole, leaves fixed in the second solution at 32° F., cut 12 μ thick, and stained in triple stain gave the most satisfactory results.

2. Sections were made of all eight varieties (series 8 and 9), 12 μ thick, a series being fixed in each fixing solution at 32° F., and triple stained. These were used for study of the method of infection.

3. Sections of the petri-dish cultures were made (series 10) for the dorsal and ventral surfaces with checks as above, the difference here being that before inoculation a small portion of the epidermis on the opposite side of the leaf had been scraped away with a sharp sterile scalpel and the cut surface immediately placed in contact with the wet toweling in the dishes. The inoculations were made on the uninjured epidermis over the cut. (The check leaves were cut but not inoculated, and none were infected.) After four days infection had occurred on all except Goldfoil. Haustoria appeared to be formed in all cases. The tests were not continued longer.

4. Another series of inoculations was made on the cut surfaces (series 11). Cuts similar to the above were made on all of the eight varieties on leaves attached to the plants, marked as before, and the plants kept under bell jars. Uninoculated check cuts were made on the same plant. In no cases were these infected.

Mycelia were formed within the mesophyll in all except Hanna, Chile, and Common Chile (Fig. 2). No reason suggests itself for the failure in these cases, since this series was repeated with similar results. These cultures were fixed, after 8 days, in the second fixative at 32° F. Sections were cut 12 μ thick and triple stained.

5. A similar series with cuts on both dorsal and ventral surfaces was made (series 13). No apparent distinction was observed. These were fixed after eight days and were treated in all respects as above.

6. No morphological peculiarities in the resistant varieties were noted from examination of slides. The size of spines and of cells varied more within the variety, depending on the age of the leaf, than between varieties. The same circumstances were found in the matter of cuticle thickness in sections stained with Sudan III, according to Priestley's (6) formula.

¹ 100 cc. 50 per cent alcohol

6.5 cc. formalin

2.5 cc. glacial acetic acid.

² 1 gm. chromic acid

1 cc. glacial acetic acid

5 gm. salts of urea

In 200 cc. distilled water.

SUMMARY

1. Susceptibility of a variety of barley to a given suitable physiological form of mildew seemed fairly constant under the conditions of culture.

2. No mean morphological differences in leaves were observed between varieties which differed in susceptibility to the given strain of mildew.

3. Injury to the host tissue by removal of epidermal cells seemed in all cases but one to reduce the resistance of the host and to allow at least sub-infection.

4. When the inoculations were made on the exposed mesophyll resistance was destroyed in all cases, and in the majority endophytic mycelium was developed.

5. The penetration of the infection threads seemed normal, a decolorized area appearing around the point of penetration in the stained sections. The collar, or membrane, formed by the host cell about the penetrating hypha, appeared clearly, while no sac or disorganized membrane was observed about the haustorium. The haustoria were typically lobed at both ends, uninuclear, and frequently many occurred in a single epidermal cell.

6. In no case was the mildew observed to enter at a stoma, whereas the mycelia commonly passed across the opening and haustoria occurred in the epidermal cells on either side.

7. The varieties of barley studied were found to have the following degrees of infection under the conditions of culture rated according to Mains' scale: Sacramento 0°, Goldfoil 0°, Common Chile 0°-1°, Chile 1°-3°, Oderbrucker 2°-4°, Hero 3°-4°, Hanna 4°, and Atlas 4°.

It seems, therefore, that the resistance of a variety of barley may be quite stable toward the given physiological form of mildew under these conditions. It seems possible then, for any given locality, to obtain a variety of barley which under ordinary conditions will be immune to *Erysiphe graminis* D.C. This resistance may be altered by circumstances affecting the vitality of the host tissue, such as a mechanical injury. It appears unlikely that this "xenoparasitism" would be able to cause the general infection of an immune variety.

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THE EFFECT OF SEED DISINFECTANTS ON SMUT AND ON YIELD OF MILLET

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Millet, *Setaria italica* (L.) Beauv., is a crop of major importance in the northern and northeastern provinces of China as well as in Manchuria. Crop disease surveys in the summer of 1925, and again in 1926, indicated that kernel smut caused by *Ustilago crameri* (Korn.) is one of the important limiting factors in the production of millet. It was not uncommon to find fields where the smut ranged from 10 to 25 per cent, and in a few cases over 50 per cent of the heads were infected. Seed from such badly infected fields together with clean seed offered excellent material for measuring the relative efficiency of different fungicides in controlling smut, the effect of these fungicides on the yield, and the losses resulting from heavy smut infection. When this study was initiated it was not known whether the remedies which had been used in the United States would be effective in China, where environmental conditions, varieties, and cultural practices are markedly different.

Experimental data concerning millet smut control and yield increases are very limited. Vasey² tried different concentrations of formaldehyde and found that a solution as dilute as 1 part of formaldehyde to 640 parts of water would kill the smut spores. He recommended either sprinkling or soaking of millet seed, using one part of 40 per cent formaldehyde in 320 parts of water. Melchers,³ in a recent paper, reported that copper carbonate at the rate of four ounces a bushel was more economical than any other dust and gave almost perfect control of millet smut. Stone⁴ states that both the formalin sprinkle and soak prevented germination of millet seed, and the dry formaldehyde also checked germination. He found that

¹ At the time these investigations were carried on the senior author was plant pathologist at the University of Nanking, Nanking, China.

The writers wish to express their appreciation to Drs. H. H. Love and C. H. Myers for helpful suggestions during the progress of these studies and to Dr. I. E. Melhus for careful reading of the manuscript.

² Vasey, H. E. Millet smuts and their control. Colo. Agr. Exp. Sta. Bul. 242. 1918.

³ Melchers, L. E. Studies on the control of millet smut. Phytopath. 17: 739. 1927.

⁴ Stone, R. E. Treatment of millet seed to prevent smut (Abst.). Phytopath. 18: 479. 1928.

Semesan and Uspulun solutions, each 0.25 per cent strength, Dupont No. 12 dust, and copper carbonate reduced smut to 1.9, 0.9, 0.6 and 1 per cent respectively. Untreated seed had 56 per cent smut.

There seems to be no published information dealing with the effect of different fungicides on the yield of smutted seed. This fact led to an investigation of this aspect of the seed treatment problem. In making a comparative study of the yield of treated and untreated seed it seemed desirable to use a number of disinfectants available and to determine their relative merits in controlling smut.

EFFECT OF SEED DISINFECTANTS ON MILLET SMUT

In the spring of 1926 a series of seed treatment experiments were begun to determine the relative efficiency of dry and liquid disinfectants. The seed used in the test was naturally smutted seed secured from a farmer whose field had 50 per cent of the heads infected with *Ustilago crameri* in 1925. The seed was collected after the threshing operation. The chemical disinfectants used in the experiments in 1926 were cold formaldehyde (1-320), Uspulun solution (3 grams a liter of water), dry Uspulun, dry Tillantin "B," and copper carbonate containing 54 per cent metallic copper. The seed was soaked two hours in the formaldehyde solution and one hour in the Uspulun solution. After soaking, the seed was spread out to dry. The rate of application for all of the dust treatments was one gram to a pound of seed, which is approximately 2 ounces a bushel. For each treatment an equal quantity of seed (60 grams) was placed in a flask, the required amount of dust added, and the flask shaken for at least five minutes or until the dust was evenly distributed on the seed. Later, 10 envelopes each containing a carefully measured quantity of seed equal to about 6 grams were prepared from each treated lot of seed. In the case of the liquid treatments the seed was weighed before treatment and after it was dried, due allowance being made for increase in size and weight.

The experiment consisted of 91 rows, each 16 feet long, arranged in such a way that every third row was a check. In this way each pair of rows from treated seed was between two rows from untreated seed. Seed from smut-free heads was included as one of the treatments. This plan made it possible to compare each treated row either with a check next to it or with an average of two checks. Each treatment was replicated 9 times, making ten in all, occurring in regular order in the plot. Border rows of common millet were planted on each end of the plot. The rows were one foot apart so that no cultivation was necessary.

The plot was planted on June 23 and harvested September 7, 1926. At harvest time each row was cut separately, the grain tied, and a label with the row number attached to it. Before threshing the total number of heads

per row was determined, together with the number of heads either partially or entirely destroyed by smut. Care was taken to prevent shattering. Each row was threshed separately and the weight of the seed determined in grams.

RESULTS IN 1926

A study of the records from this plot showed that the checks were quite uniform in yield, which indicated that the soil did not vary greatly in fertility. In computing the results, each treated row was paired with its adjacent check and a comparison made between each 10 pairs for a particular treatment. By the use of "Student's" method the reliability of the results was measured in terms of odds. A summary of the results for 1926 are presented in table 1.

The acre yield data shown in table 1 as well as the data on percentages of smut are averages of 10 in every case.

None of the treatments completely eliminated smut. This may be due to insufficient quantity of dust in the case of the dry treatments, although the formaldehyde soak treatment was even less effective than dry Uspulun and Tillantin "B." Furthermore, the soil on which the plot was planted had never grown millet before. In only three cases, however, was the percentage of smut in a treated row greater than its nearest check and all of these three were in the rows treated with Uspulun solution. Tillantin "B" and dry Uspulun each proved slightly more effective in controlling smut than any of the other disinfectants.

RESULTS IN 1927 WITH NATURALLY SMUTTED SEED

In the spring of 1927 the test was continued in much the same way as in the previous year except that the rows were 10 feet long and there were two separate plots planted, one with naturally smutted seed of the same source as in 1926, the other with seed artificially smutted. In the first plot, only three dusts were used, namely Uspulun, Tillantin "B," and copper carbonate, each at the rate of 2 ounces a bushel. A check was planted every fourth row so that the three treatments were between two checks. Nineteen replications were made, giving 20 rows of each treatment. The arrangement in planting was such that the row treated with Uspulun always occurred between the rows treated with copper carbonate and Tillantin. In computing the results each treated row was paired with an average of the two nearest checks and odds determined by "Student's" method. The results of this plot are shown in table 2.

The soil used in 1927 for the first plot was much more fertile than that used in the previous year. Differences were more uniform and with a

TABLE 1.—*Effect of certain treatments of naturally smutted millet seed on smut and on the yield. Nanking, China, 1926*

Treatment	Smutted heads (per cent.)		Difference in per cent	Acre yields (bu.)		Increase due to treatment (bu.)	Gain in per cent	Odds
	Treated	Not treated		Treated	Not treated			
Selected heads	4.7	30.9	26.2	24.0	17.6	6.4	36.3	344-1
Formaldehyde	3.3	21.4	18.1	22.9	18.0	4.9	27.2	163-1
Uspulun solution ..	15.2	21.4	6.2	19.7	18.0	1.7	9.4	10.9-1
Uspulun (dry)	3.0	30.1	27.1	22.3	18.0	4.3	23.9	81-1
Copper carbonate ..	4.1	30.1	26.1	21.8	18.0	3.8	21.1	22.5-1
Tillantin "B"	2.7	27.1	24.4	23.9	18.4	5.5	29.9	908-1
Average of all checks	26.8			18.0				

TABLE 2.—*Effect of certain dust treatments of naturally smutted millet seed on smut and on yield. Nanking, China, 1927*

Treatment	No. heads per row	Smutted heads (per cent)		Difference in per cent	Acre yield (bu.)		Increase due to treatment (bu.)	Gain in per cent	Odds
		Treated	Not treated		Treated	Not treated			
Uspulun (dry)	238	3.4	20.6	17.2	48.6	36.9	11.7	31.7	4999 to 1
Copper carbonate ..	213	2.6	20.6	18.0	44.7	36.9	7.8	21.1	2499 to 1
Tillantin "B"	274	4.7	20.6	15.9	46.0	36.9	9.1	24.7	2499 to 1
Average of all checks	197		20.6			36.9			

larger number of replications the odds are greater. The yield of the checks was twice as great as in 1926, even though the percentage of smut is not much less. None of the treatments completely eliminated smut but all reduced it to less than 5 per cent as compared with 20.6 per cent in the checks. Dry Uspulun allowed 0.8 per cent more smut than copper carbonate but gave the largest increase in acre yield, namely 11.7 bushels. Dry Tillantin gave the poorest control of any of the treatments but produced an increase of 9.1 bushels an acre, which is 2.6 less than for Uspulun and 1.3 more than for copper carbonate.

RESULTS IN 1927 WITH ARTIFICIALLY SMUTTED SEED

In the spring of 1927 the quantity of naturally smutted seed available was insufficient for all of the tests. Accordingly a sample of nearly smut-free seed was inoculated with smut spores two years old. The smutted heads were rubbed between the hands, put through a fine screen and the resultant smut powder was scattered evenly over the seed by first placing a quantity of seed in a tea strainer and then adding the smut powder. A thorough shaking of the mixture distributed the smut spores over the seed. This seed was then divided into several lots and treated with four different dusts and two liquids. The dusts were used at the rate of 2 ounces a bushel. The two liquid treatments, Uspulun and Tillantin-Hoesht, were made up by dissolving 3 grams of the dust in a liter of water. The seed was soaked one hour and then thoroughly dried.

The planting plan was the same as for 1926, that is, two treated rows were placed between a pair of checks. The rows, however, were only 10 feet long, with nine replications of each treatment. This plot was planted at the same time as the one with naturally smutted seed. The results of this test are given in table 3.

The results presented in table 3 show that all of the treatments reduced the smut to less than 1 per cent, but the amount of smut in the checks was low as compared with that in plants grown from naturally smutted seed.

In the two years' work with millet smut eight different seed disinfectants were used on both naturally and artificially smutted seed. The old standard formaldehyde treatment proved just about as effective in controlling smut as any other treatment used, but it did not completely eliminate the disease on badly smutted seed. Uspulun and Tillantin "B" each used as dusts gave approximately the same control on the two kinds of seed. The average percentage of smut for the two years on naturally smutted seed was 3.2 when treated with Uspulun, 3.7 for Tillantin "B," and 3.4 for copper carbonate. If we make the comparison between the three dusts on the basis of the smut reduction due to treatment we find that there is little difference. The average percentage of smut reduction for the two

TABLE 3.—*Effect of certain dry and liquid treatments of artificially smutted millet seed on smut and on yield.*^a Nanking, China, 1927

Treatment	No. heads per row		Difference	Smutted heads (per cent)		Difference in per cent		Acre yields bu.		Gain in bu.	Gain in per cent
	Treated	Not treated		Treated	Not treated			Treated	Not treated		
Uspulun (dry)	219	187	32	0.9	6.8	5.9		41.6	40.4	1.2	3.0
Uspulun solution	214	187	27	0.6	6.8	6.2		41.1	40.5	0.6	1.5
Tillantin- Trochenbeize ..	208	212	-4	0.3	6.8	6.5		44.2	41.3	2.9	7.0
Tillantin- Nazzbeize	208	212	-4	0.2	6.8	6.6		40.1	41.3	-1.2	-2.9
Tillantin-“B” ..	219	200	19	0.9	6.8	5.9		43.2	42.1	1.1	2.6
Tillantin-Hoesht solution	196	200	-4	0.4	6.8	6.4		41.9	41.7	0.2	0.5
Average of all checks	198			6.8				41.2			

^a All of the materials used in this test were furnished by the China-Export-Import-Bank Co. Ltd., of Shanghai, China.

years on naturally smutted seed is 22.2 for Uspulun, 22.1 for copper carbonate, and 21.2 for Tillantin "B." Apparently the three dusts are about equally effective in controlling smut. It is possible that a heavier application in each case would come nearer to a complete control.

Uspulun solution gave practically as good control as any other material on artificially smutted seed, but in one year's test it was very ineffective when used on naturally smutted seed. Three other disinfectants were used only on artificially smutted seed, namely Trochenbeize and Nazzbeize as dusts and Tillantin-Hoesht as a solution. Each of these three gave slightly better control than any of the other treatments in one year's trial.

EFFECT OF FUNGICIDES ON THE YIELD OF SMUTTED MILLET SEED

The results presented in tables 1, 2, and 3 show some variation in the yields from smutted seed treated with different fungicides. In two instances the difference in the yield due to liquid and dry Uspulun could be attributed to more effective control of smut. For example, in 1926 the increase in yield following treatment with Uspulun solution was 1.7 bushels an acre with 15.2 per cent smut on the treated rows. Dry Uspulun reduced the smut to only 3.0 per cent and increased the yield 4.3 bushels an acre with significant odds. Contrary to the above situation the yield increases in most cases following treatment cannot be accounted for on the basis of smut control entirely. The average increases in yield for two years on badly smutted seed treated with dry Uspulun, Tillantin "B," and copper carbonate are 8.1, 7.3, and 5.8 bushels an acre respectively. These increases expressed in percentages are 27.8, 26.8, and 20.9 in the order mentioned above. The average percentage of smut after treatment as well as the percentage of smut reduction due to treatment was nearly the same in each case. In the test in 1927 where 19 replications were made on better soil than in 1926 these variations are greater than an average for the two years. Uspulun reduced the smut 17.2 per cent but increased the yield 31.7 per cent, Tillantin reduced the smut 18.0 per cent and increased the yield by 24.3 per cent, whereas copper carbonate reduced the smut 15.9 per cent and increased the yield only 21.1 per cent. It seems, according to these tests, that Uspulun and Tillantin must have some different effect than copper carbonate, and Uspulun even more effect than Tillantin. One possible explanation is injury which could be more readily charged to the copper carbonate than to either of the other dusts because of the smaller increase in yield. Another possible factor is that other diseases on the seed may be controlled by one dust more effectively than by another.

A species of *Phoma* is commonly found on millet seed in China. This fungus unless controlled could possibly cause some seedling blight. The oospores of *Sclerospora graminicola* also occur commonly on millet seed

from north China. Whatever the cause of these variable results it is evident that the value of a seed disinfectant cannot be measured entirely by its effectiveness in controlling smut. Yield increases or decreases must also be determined.

The effect on yield of the other treatments used in 1927 on artificially smutted seed was not so easily determined. The soil was variable in fertility as evidenced by great differences in the yield of individual check rows not far removed from each other. In addition the untreated seed was not badly smutted. Before any definite conclusions can be drawn it will be necessary to test them for at least one more season. It may be of some significance, however, that only one treatment, namely Tillantin-Nazzbeize, reduced the yield.

COMPARATIVE YIELD OF NEARLY SMUT FREE AND SMUTTED SEED

In order to determine the effect of smut on the yield of grain it is necessary to compare the yields of disease-free and smutted seed from the same source and of the same strain or variety. One method is to inoculate some clean seed artificially and then compare the yield of it with smut-free seed. In any such test the percentage of smut as well as the yield of each sample must be determined. In this experiment another method was used. Fifty smut-free heads were selected at random from a field in which 50 per cent of the heads were partially or entirely infected with smut. These heads were gathered just before harvest in 1925, the seed was threshed and stored separately and used in planting the following spring. From the same field a few pounds of seed were secured after the grain was threshed, at which time all of the seeds were almost black owing to the presence of spores of the millet smut fungus. In the spring of 1926 seed from these two samples were planted in rod rows, which were repeated nine times. Immediately after the rows were harvested, each bundle was labeled and a count made of the total number of heads together with the number of smutted heads. The yield of each row was determined in grams and later computed in terms of bushels an acre. The planting of this test was made a part of a seed treatment study given in table 1. By reference to the table it will be seen that the seed from selected heads had 26.2 per cent less smut than the untreated checks. The checks yielded 26.6 per cent less grain than the seed from selected heads. From this it is evident that in 1926, 26.2 per cent smut reduced the yield by 26.6 per cent. It is worthy of note that although seed from heads selected from a badly infected field had slightly more smut than any treated seed with the exception of the seed treated with Uspulun solution, yet the yield increase was greater than in any of the treatments. Furthermore, this test indicates that most of the smut infection takes place during the threshing operation and for that reason seed

selection before harvest would be of great help in the control of smut, a practice which could easily be carried out by Chinese farmers.

SUMMARY

Tests were carried on at Nanking, China, in 1926 and 1927 with nearly smut-free and badly smutted millet seed.

Formaldehyde, dry Uspulun, copper carbonate, and dry Tillantin "B" were all about equally effective in controlling smut on naturally smutted seed, although none of them reduced the smut to less than 2.6 per cent. The checks showed an average of 26.6 per cent in 1926 and 20.6 in 1927. Uspulun solution gave poor control.

Dry Tillantin "B," Uspulun, Tillantin-Trochenbeize, Tillantin-Nazzbeize, and liquid Uspulun and Tillantin-Hoesht, all reduced smut to less than 1 per cent in comparison with 6.8 per cent smut on untreated, artificially smutted seed.

On badly infected seed, all of the treatments used increased the yield, the increases ranging from 1.7 to 11.7 bushels an acre, depending on the fertility of the soil and the treatment used. Dry Uspulun and Tillantin "B" gave consistently larger increases in yield than copper carbonate.

Smut-free heads selected before harvest from a field badly infected with smut produced a crop with 4.7 per cent smut, which was 26.2 per cent less than that in the crop produced by seed from the same field collected after threshing. The increase in yield from seed of selected heads was 26.6 per cent.

Where a small acreage is planted on each farm as in China, head selection before harvest in combination with seed treatment should prove worth while.

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CONTROL OF BUNT OF WHEAT IN NEBRASKA¹

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INTRODUCTION

The application of copper carbonate by Darnell-Smith (1) in Australia in 1917, supplemented by the later studies of Mackie and Briggs (6) in California, Heald and Smith (4) in Washington, and others, for the control of stinking smut or bunt of wheat, opened up a new era in the control of this altogether too common and serious disease. The use of copper carbonate dust has rapidly spread over the entire country so that it has now become an established treatment for bunt of wheat. At about this same period there were introduced into this country a number of organic mercury compounds for the control of seed-borne diseases, including bunt in wheat. A number of these dusts, recommended for the control of bunt, were included in the tests.

In the main the many results obtained by the various investigators in the several states have shown a very uniform and marked control, especially with the copper carbonate dusts. Occasionally, however, complaints have been made by growers that the control of bunt has not always been so satisfactory as had been anticipated. This has been especially true in certain sections of Nebraska. In order to determine what factors were responsible for the varying results obtained by farmers in Nebraska, a series of tests was begun in 1923 and continued through 1927 at two places, the Station Farm at Lincoln and the North Platte Substation, located 250 miles west of Lincoln. At North Platte wheat is grown under dry-land conditions, typical of much of the upland of the western part of the state outside of the sand-hill sections.

METHODS

The seed wheat used was Nebraska No. 60, a very uniform strain of Turkey developed by the Department of Agronomy. The original bunt inoculum was obtained through the courtesy of F. N. Briggs. Sufficient inoculum was gathered from the test plats each year for the following year's test, so that probably the same smut strain was carried through the period of the experiments. The same spore load, 5 grams of inoculum to 100

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² The writer wishes to acknowledge his indebtedness to G. F. Sprague, for conducting the experiments at the North Platte Substation, and to T. A. Kiesselbach and his assistants for caring for the plats at Lincoln.

grams of wheat, was applied to the grain each year. Heald (3) has found that this spore load is necessary to produce maximum smutting. Uniform methods of applying the dust compounds were also employed. All the compounds were from the original containers obtained at the beginning of the tests. During the course of these experiments the following compounds have been used one or more years: copper carbonate, Corona Copper Carb, copper stearate, colloidal copper, Jabonite, Semesan, Bayer dust, Corona 40-S, Corona -620, Seed-o-san (white), Seed-o-san (pink), and formaldehyde. All compounds of unknown composition were discontinued after the first year and are not included in the discussion. All dusts were applied at the rate of 2 ounces a bushel. Formaldehyde was used in the concentration of 1-320, and the seed soaked for 15 minutes. Through the use of the same wheat, same source of inoculum, same spore load, same dust compounds, and the same rate and method of application, the tests were made as uniform as possible.

One-half of each lot of treated seed was retained for planting at Lincoln while the other half was sent to North Platte. The plats varied somewhat in their location from year to year at Lincoln in order to conform with rotations, but were on the same type of soil. This was likewise true of the plats at North Platte, where the seedings were made in connection with other wheat experiments. While the soil varied somewhat in the two localities, the hydrogen-ion reaction was about the same. The only variables in these experiments were the date of planting and the resulting environmental and soil factors encountered by the germinating seeds and growing seedlings in the two localities.

The percentages of smut in the plats given in the tables are based on head counts. For two years counts were made on infected culms also, but no appreciable differences between the two methods of counting were noted. The plats were 1/40th of an acre and were run in either duplicate or triplicate.

RESULTS OF THE TESTS AT NORTH PLATTE AND LINCOLN

At the time the plats were planted, 200 additional seeds of each lot were planted in rod rows, and a count was made in October of the resulting plants and another in April of the number of plants which survived the winter. This was done to determine whether any of the treatments increased or decreased germination and the subsequent survival of the plants. No appreciable differences were noted with any of the treatments with respect to germination, stimulation, and winter survival, with the exception of those treated with formaldehyde, which gave a consistent and lower percentage of germination.

In table 1 are listed the results obtained with the different treatments at North Platte for the years 1923-24, 1924-25, and 1926-27. No bunt was found in any of the plats during the 1925-26 season owing to the low percentages of germination and survival, primarily caused by the dry, windy, and cold season. The average percentage of germination in the rod rows of all treatments in 1925-26 was only 56.5 per cent, while only 15.7 per cent of those which germinated survived, as contrasted with 80.4 per cent germination and 72.1 per cent survival the previous year.

It will be noted that the treatments were effective the first season when only 11.4 per cent of smut was found in the untreated controls. Relatively similar results were obtained in 1926-27, but again only 13.8 per cent of smut was found in the untreated controls. However, in 1924-25, 46.3 per cent of smut occurred in the untreated controls and no treatment was successful in completely controlling bunt; even the formaldehyde-treated seed yielded 6.6 per cent of smut. Some of the treatments, however, greatly reduced the amount of bunt compared with the untreated controls.

The data obtained for three years at Lincoln with the various treatments are also listed in table 1. During the season 1924-25 all dust compounds gave very good results, with the untreated controls showing approximately 15 per cent of bunted heads. During the next two years the untreated controls had a high percentage of infection, approximately 41 and 40 per cent respectively, with considerable bunt showing in most of the treatments

TABLE 1.—*The percentage of bunt in the various tests at the North Platte Substation and at the Station Farm, Lincoln (1923-27)*

Treatments ¹	1923-24	1924-25		1925-26	1926-27	
	North Platte	North Platte	Lincoln	Lincoln	North Platte	Lincoln
	(per cent)	(per cent)	(per cent)	(per cent)	(per cent)	(per cent)
Untreated control	11.4	46.3	14.9	41.2	13.8	39.7
Copper carbonate	0.3	8.6	0.7	7.7	0.08	1.9
Corona Copper Carb	0.4	13.1	1.03	9.1	3.7	5.0
Colloidal copper	—	—	—	23.7	—	—
Copper stearate	—	30.0	1.73	26.8	—	—
Jabonite	—	—	—	—	1.1	11.8
Semesan	0.4	18.7	0.8	11.9	2.2	8.7
Bayer dust	—	—	—	30.3	2.4	18.9
Formaldehyde	—	6.6	0.01	7.0	0.9	2.0
Non-inoculated control	—	2.5	0.06	8.2	0.0	0.6
Date of planting	Sept. 23	Sept. 24	Oct. 16	Oct. 10	Sept. 18	Oct. 9

¹ All seed was inoculated similarly except as noted.

as contrasted with the first year's trials. Even formaldehyde failed to check bunt completely during the season of 1925-26.

INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON BUNT INFECTION

It is clearly evident that although the seed was handled uniformly the effectiveness of the treatments varied not only from year to year in the same locality, but also differed widely during the same season at the two points in the state. The results indicate that when a small to moderate infection occurred in the untreated controls, most of the dust treatments were very effective in controlling smut, whereas when a high percentage of smut was recorded in the untreated controls, these treatments, while giving good control in some instances, were not on the whole nearly so effective. It appears that under conditions favoring smut infection a number of dust compounds for some reason or other are not wholly effective in smut control and this fact may account for some of the complaints received from farmers of the unsatisfactory results obtained with some of these treatments. In order to deduce a possible explanation of these results a brief account of the nature of smut infection and its relation to environmental conditions is presented.

Woolman (9) has found that the fungus enters the epidermis of the coleoptile of both susceptible and resistant wheat plants but that in resistant varieties it penetrates no further. The time required by the fungus to pass into the permanent tissues of the plant he states to be 10 days.

Thomas (8) also reports that the coleoptile is the region of attack, and that after entrance is effected the organism continues to develop within and at the expense of the wheat plant. He also states that infection takes place coincidentally with the germination of the seed and that naturally the period of susceptibility of the seedling is short, as the germinating smut spore can survive for only a limited period in the soil—usually not longer than a month.

According to Percival (7) the coleoptile functions chiefly as a protective cover to the young foliage leaves during their upward growth through the soil and is made of tissues of a simple character. He finds that the coleoptile does not remain active, but gradually shrivels up after the first leaf starts to uncurl.

Judging from the above accounts, infection of the seedling takes place through the coleoptile and the period of susceptibility extends from the inception of germination until the coleoptile becomes functionless. When the extent of the life of the plant is considered, this is an extremely short period. Apparently the environmental conditions do not have any influence on the development of the fungus once the permanent tissues are reached,

so that we are concerned only with the life of the seedling from the inception of germination until the coleoptile shrivels up.

Several experiments were carried out with a number of winter wheats at different controlled soil temperatures to determine first the percentage of germination and second the time required for the seedlings to emerge. All seed was planted at the same depth in a soil with a constant moisture (35 per cent dry weight 62.8 M. H. C.) which was found to be near the optimum. The results of tests are given in table 2.

TABLE 2.—*The influence of various soil temperatures on the percentage of germination and the time required for the emergence of the seedlings of a number of winter wheats*

Soil temperature (degrees C.)	Germination (per cent)	Time for emergence (days)
5	73	22
10	77	12
15	81	8
20	78	4
25	75	3
30	65	2

During these tests it was noted that the hypocotyl was very much elongated at 25° C. and much more so at 30° C. When the containers in which the seeds were planted and held at 30° C. for one week were transferred to 15° C., some seeds germinated, indicating that not all inhibited seeds were killed at the higher temperature.

The striking point brought out by the above data is the fact that there is not much variation in the percentage of germination at the soil temperatures of 5° to 25° C., when the time element is eliminated. The highest percentage of germination was obtained at 15° C., which appears to be near the optimum for germination of the wheat varieties tested. However, when the time for emergence is considered, only 2 days are required at 30° C., while at 5° C. 22 days are required. Even at the optimum soil temperature (15° C.) for germination, 8 days elapse before emergence. Thus the time required for emergence is an important factor, as the infection court, namely the coleoptile, is receptive to infection over a greater period of time at the lower temperatures.

Woolman and Humphrey (11) find that the optimum temperatures for spore germination lie between 18° and 20° C., while the minimum and maximum temperatures respectively are 0° to 1° C. and 25° to 29.1° C. In table 3 the time required for the first evidence of spore germination, as

adapted from the data obtained by Woolman and Humphrey (10 and 11) and others cited by them, is compared with the time necessary for the seedlings to emerge at the same temperatures as shown in table 2.

TABLE 3.—*The time required for seedling emergence at various temperatures compared with the time required for the first sign of spore germination, as adapted from the data of Woolman and Humphrey*

Temperature (degrees C.)	First sign of spore germination (days)	Time for seedling to emerge (days)
5	12	22
10	5	12
15	3-4	8
20	2	4
25	No response?	3
30	No response	2

Thus at the higher temperatures the spores are unable to germinate at all, while at 20° C. and lower the spores germinate in less time than is required for the seedlings to emerge. In all probability the coleoptile develops very slowly at the lower temperature and so provides an excellent infection court for a much more extended period than at the higher temperatures.

Two tests were made to determine the relation of soil moisture to seed germination on several winter wheats. The seeds were all planted at the same depth and a soil temperature of near 20° C. was maintained during the course of the experiment. Soil having a moisture equivalent of 15 was used. Four different soil moistures were tested, as noted in table 4.

TABLE 4.—*The percentage of germination of a number of winter wheats at different soil moistures*

Soil moisture (dry weight in per cent)	Germination (per cent.)
10	68
14	90
18	78
22	79

The optimum soil moisture for germination of the seed tested appears to be at 14 per cent with a moisture equivalent of 15.

Hungerford (5) finds under controlled conditions in the greenhouse "that low soil temperatures and a fairly high percentage of moisture in

the soil are both conducive to stinking smut infection. . . . The highest percentage of infection was secured at temperatures ranging from 9° to 12° C. and in soil containing 22 per cent moisture and with a moisture equivalent of 20.7." He found further "that an exceedingly high percentage of moisture in the soil seemed to inhibit infection." The moisture content of the soil in which he obtained the highest percentage of infection is only slightly higher than that in which the writer found the highest percentage of germination. In this connection Woolman and Humphrey (11) state that the germination of the spores in a saturated soil is inhibited, the spores losing their viability in about 35 days. They regard the optimum soil moisture content for infection in the basaltic soil of the Palouse country to be between 16 and 30 per cent. Therefore it seems probable that the soil moisture best suited for the germination of the seed is the one most conducive to spore germination and subsequent infection. Extremely low and high soil moistures seem to inhibit the germination of the spores to a greater extent than they do the wheat seed. On the whole it appears that soil moisture is not so important a factor in smut infection as soil temperature.

Hungerford (5), Woolman and Humphrey (11), Faris (2), and others have shown that soil temperatures below 20° C. are very conducive to bunt infection. Hungerford obtained the highest percentage of infection under control conditions at temperatures from 9° to 12° C. Likewise, Faris reports maximum infection at temperatures of 5°, 10°, and 15° C. As pointed out previously, spore germination decreases very rapidly above 20° C., which may account for the small amount of bunt reported by these workers at temperatures above this point. From these studies it is reasonable to conclude that soil temperature is the most important factor involved in bunt infection in the field, providing smutty seed is planted.

Unfortunately soil temperatures were not recorded at Lincoln and North Platte during the years the tests were made. However, on comparing the air temperatures for these years it is found that general analogies can be drawn from these data. During the fall seasons of 1923 and 1926 at the North Platte Station the rather low percentage of infection in the untreated plats can be correlated with temperatures higher than those occurring in 1924, when the percentage of bunt in the untreated controls was almost four times as large. The entire fall and winter season of 1925 was so unfavorable to the growth of the wheat itself that no bunt developed in any of the plats. At Lincoln rather higher temperatures prevailed during the infection period in 1924 than 1925 and 1926, the percentage of bunt in the untreated control plats being three times as large the last two years.

The mean temperatures during the infection period in 1924 at North Platte were much lower than those recorded at Lincoln, which accounts for

the high percentage of infection (46.3) in the untreated control plats at North Platte and the rather low percentage of infection (14.9) at Lincoln, although the same inoculated and treated seed was used in both localities.

The data show that when the incidence of bunt infection is low, most of the seed treatments tested are more or less effective; whereas when the percentage of infection is high, the less effective treatments are easily recognized. Under these conditions the effectiveness of all treatments is lowered. This was true even with formaldehyde. The only explanation that can be offered is that, when environmental conditions favor maximum bunt infection, the dust treatments are not completely effective in preventing some of the bunt spores from germinating and entering the plant tissues. In testing dust treatments for the prevention of bunt, they should be carried out not only in different localities but also under conditions which will produce maximum infection. In this way the less effective treatments can be more easily eliminated. The wheat grower should be urged to plant treated seed, as nearly at the normal planting date for his locality as possible, to avoid conditions favoring bunt infection.

SUMMARY

1. No appreciable differences were noted with any of the dust treatments with respect to germination, stimulation, and winter survival, except in the tests with formaldehyde, which gave a consistent and lower percentage of germination.

2. Formaldehyde was the most effective treatment under all conditions. Of the dust treatments, applied at the rate of 2 ounces a bushel, copper carbonate gave the most effective control. Corona Copper Carb was slightly less effective.

3. None of the other copper or organic mercury compounds showed as great a degree of control as the copper carbonates, especially under conditions favorable for maximum bunt infection.

4. It appears that the effectiveness of all treatments tested is lessened in varying degrees under environmental conditions favoring maximum bunt infection.

5. Treated seed should be sown as nearly at the normal date of planting for the locality as possible.

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ENDOSEPSIS AND ITS CONTROL IN CAPRIFIGS

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In recent years the calimyrna¹ fig industry in California has been seriously threatened by the increasing prevalence of three diseases of the fruit: smut (7), souring (3), and endosepsis (1), which during the past three years have caused the growers an estimated annual loss of well over 50 per cent of their crop. In addition to this loss of crop the hand-sorting of both fresh and dried fruits which has become necessary because of these diseases has so raised the cost of production that the industry in some districts has ceased to be a profitable one.

Of the three diseases named above, endosepsis is by far the most important, being easily responsible for 80 per cent of the cull pile. This disease was formerly recognized under the various names of "brown rot," "pink rot," "soft rot," and "eye-end rot," terms that are strikingly descriptive of various phases and symptoms of the disease but somewhat confusing because of their multiplicity and quite undesirable because of their use in connection with specific diseases of other fruits. It is now known under the name of "endosepsis," a Greek term which means internal rot. Caldis, who described and named the disease (1), has worked out its transmission and etiology and named the causal organism *Fusarium moniliforme* var. *fici* Cald.

This fungus grows in the cavities of caprifigs where it sporulates abundantly. When the caprifying insects, *Blastophaga psenes* L., emerge from the galls they pick up spores on wings and other body parts and carry them to other caprifigs or to the calimyrnas, where they germinate and produce the endosepsis.

We have no authentic report of endosepsis occurring in calimyrnas prior to 1920 (8). Since then the disease has been found in every fig-growing section in California, with losses estimated at from 20 to 90 per cent (1).

Since it has been shown that the internal rot is transmitted from the caprifigs to the calimyrnas by the caprifying insect, it seems logical to assume that if the causal organism were eliminated from the caprifigs there would be no spores for the insects to pick up and the rot in the calimyrnas would be automatically controlled. This paper, as the title indicates, deals mainly with the various manifestations of the causal organism in caprifigs and its eventual elimination from them.

¹ The calimyrna is the Lob Injir variety of Smyrna grown in California.

INTERRELATION OF FIG, FUNGUS, AND INSECT

Technically the insect *Blastophaga psenes* L. is considered to be parasitic on the caprifig, but actually the relation is a truly symbiotic one, as neither can properly develop, mature, or propagate without the other. The relation between the fungus and the caprifig is one that might be called inactively parasitic on the part of the fungus and rather passive on the part of the host. The fungus does not invade actively growing or healthy tissues but subsists on non-living material such as floral and other parts of the fig that have become inactivated through age or been killed by ovipositing blastophaga or other agents, and on the dead bodies of the insects themselves. The tissues of the fig are, therefore, not extensively invaded until the fruit is ripe; hence it develops and matures in normal manner and season with no apparent ill effect due to the presence of the fungus. From the standpoint of the fungus the triple alliance (fig, fungus, and insect) is a most favorable one, especially to propagation and distribution; the comparatively dry meat of the fig, though unfavorable to much vegetative growth, is an excellent medium for the production of spores, and the large numbers of insects present at the time of maximum sporulation furnish a convenient means of ready transportation.

METHODS OF DISSEMINATION

Climate or temperature is the most important factor in the maturing of caprifigs, much more so than in the calimyrnas. Hence in sections where caprifigs mature late the grower frequently imports profichi from districts where caprifigs mature early in order to get the first part of his calimyrna crop properly caprifigged, and vice versa. Likewise after winters when local frost has destroyed the hold-over capris, reestablishment of the blastophaga is brought about by importation of mamme figs from sections where the frosts were less severe.

Each caprifig contains an average of four hundred insects (4). If infection had taken place in only one mamme fig it would be theoretically possible, assuming that each insect went to a different fig, to have as many as four hundred infected figs in the following crop (profichi) and in the succeeding crop (mammoni) as many as one hundred and sixty thousand (160,000). In nature this phenomenal increase from 1 to 160,000 in one year does not occur for the following reasons: (1) Usually several insects enter the same fig, thus decreasing the number of figs entered and infected; (2) The bulk of the profichi crop being used for pollinating the calimyrnas is removed from the capri trees, and thus, though they cause rot in the edible figs, they are prevented from causing further increase in the number of caprifigs infected. (3) Not all insects emerging from an infected fig

carry the fungous spores for the reason that sporulation sometimes, in certain varieties and under certain conditions, does not begin until one or two days after the insects have begun to issue. If, however, such figs have frost lesions or other injuries that give rise to dead tissues in the fig, then sporulation will be abundant before emergence and practically all the insects issuing will become infected. (4) Not all insects find their way into figs. The practice of intersectional distribution of caprifigs, the ability of the insects to fly considerable distances combined with the rapid natural increase in the disease once infection has taken place appear to offer an adequate explanation of the almost epidemic rapidity with which endosepsis has increased and spread.

CONTROL OF ENDOSEPSIS IN CAPRIFIGS

In view of the fact that the structure of the fig does not allow ready application of fungicides, it seemed that the only way to effect control of endosepsis would be to find non-infected caprifigs, propagate them in isolated places where all other caprifigs had been removed and in this manner establish a colony of blastophaga free from the fungus. Several difficulties attended this method of procedure. In the first place clean figs would have to be sought for by a hit-and-miss method as there are no external symptoms of the disease in caprifigs, and with the high percentage of infection clean figs were seldom found. In the second place, the only way definitely to determine the presence or absence of the fungus in a fig is to culture it. During the process of culturing the insects are killed and therefore valueless for propagation. For several years Dr. P. D. Caldis and others tested caprifigs from various parts of the state in an effort to find and establish a clean colony of blastophaga. Though a few individual figs were found that apparently did not carry the fungus, no colony of clean blastophaga was successfully established.

When the writer commenced work on control of endosepsis in July, 1926, it occurred to him that by means of a hypodermic syringe it might be possible to inject into the cavities of caprifigs a fungicide that would kill the fungus without injuring either the fig or the insects still in the galls. The preliminary experiments carried on with mammoni figs gave very promising results (6), showing that by the injection method clean figs and insects could be produced. The injection method, however, had some very serious limitations that made it undesirable for use on a large scale. The operation requires considerable skill and very great care, making it a slow and tedious affair. Because of internal structure (solid center) most varieties of caprifigs can not be treated by this method. To be effective, injection must be done over a short period of time, preferably within six or eight weeks after the figs have been caprifigged. And finally, when inject-

ing a large number of figs on a tree, a few might easily be overlooked and left untreated to start infection over again. Obviously a method that would overcome these difficulties would be desirable. As has been pointed out before, in the mature caprifig the fungus is too deeply imbedded in the tissues of the host to be reached and killed by a fungicide, but it was thought that sterilization of the fig cavity and gall surfaces would not only kill all superficial mycelium and spores but would also delay further sporulation until most of the insects had emerged. The following experiment was devised to test this assumption. Ninety mature mamme figs were selected from which the insects were either actively emerging or just about to emerge. These figs were cut into halves. One half of each was treated by immersion in 2 per cent Semesan for 15 minutes and the other half was kept as an untreated control. The halves, both treated and untreated, were placed separately in sterile bottles for observation and examination. In addition 20 whole untreated mamme figs were placed in sterile bottles to see if cutting into halves would affect the percentage of infected insects. As the insects emerged, about 10 from each bottle were placed on nutrient culture media each day for seven days to determine their sterility. The results are shown graphically in figure 1.

The two upper curves (1 and 2) in figure 1 show a considerable difference in infection of insects emerging from whole and cut figs. The reason is probably this: that all insects leaving a whole fig must pass through the eye, which, in the mamme, as well as in all other caprifig figs, is the principal place of infection because the entering blastophagas usually tear off their wings and leave them wedged between the scales surrounding the eye of the fig. These wings, bearing numerous setae, are almost ideal mechanisms for carrying spores of various kinds.

The two lower curves (3 and 4) show that in the dipped figs fully 90 per cent of the insects emerge before the fungus has developed enough to infect them. They also appear to show by their overlapping that figs so treated must be destroyed within five days to obviate reinfection from insects emerging later. Under the conditions of the experiment both temperature and humidity were higher and more constant than in the field. Since these conditions hasten the emergence of the blastophaga and also the development and sporulation of the fungus, we would under field conditions expect curves 3 and 4 to be altered somewhat in shape but very little in their relation to one another. In general, results seem to indicate that the objectionable features attending injection can be eliminated by use of the cutting and dipping method.

On the basis of results obtained in the preliminary experiments it was decided to test the two methods on a larger scale in the field. Caprifig-

groves were selected at various places in the state on the basis of their isolation from other capri trees, to minimize the danger of insects flying in from infected caprifigs. The groves were located as follows: Fresno, Calif. (12

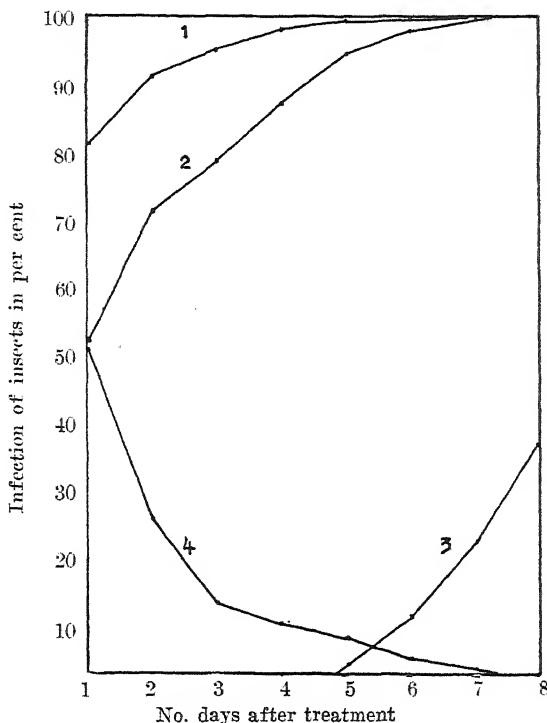


FIG. 1. The relation of rate of emergence to rate and amount of endosepsis infection of *Blastophaga* from treated and untreated caprifigs.

Curve 2. Percentages of infected insects from 20 whole, untreated mamme figs.

" 2. Percentages of infected insects from 90 halves of untreated figs.

" 3. Percentages of infected insects from 90 halves of treated figs.

" 4. Rate of emergence of insects. This appeared to be practically the same for treated and untreated figs.

large capri trees); Merced, Calif. (10 medium-sized capri trees); Madera, Calif. (30 large capri trees). At these places all the hold-over figs (mamme) were removed and destroyed. At a fourth place located at Davis, Calif. (12 capri trees), no treatments were given and this place was chosen as a control. At two other places near Fresno several thousand mamme figs were injected with 2 per cent Semesan during January, 1927. When these figs became mature they were picked and used to effect recolonization at

Fresno and Merced. For recolonization of the profichi at Madera the dipping method was employed. Mature mamme figs were cut, dipped in 2 per cent Semesan for 15 minutes, and then hung in the trees in small baskets. At intervals of four days these figs were removed and burned and replaced by others.

As soon as the profichi figs began to ripen representative numbers of them were brought to the laboratory and tested by microscopic and cultural methods for endosepsis. The results are given in table 1.

TABLE 1.—Results from culturing profichi caprifigs with insects from treated mamme figs

Place (California)	Method of treatment	No. figs cultured	No. figs infected	Percentage infected
Fresno	Injection	400	4	1.0
Merced	Injection	178	0	0.0
Madera	Dipping	208	0	0.0
Davis	None	100	100	100.0

The data in table 1 indicate that *Fusarium moniliforme* var. *fici* Cald. may be eliminated from caprifigs either by injecting Semesan into green ones or by cutting mature ones into halves and immersing them in the fungicide.

Though the cutting and dipping method appears to be quite effective, it has two drawbacks that would seriously hamper its application on a large scale. (1) the figs must be left on the trees until they are mature so as to enable the bulk of the insects to emerge within a few days after disinfection. Under this condition many insects escape prior to the treatment, making thorough control impossible. If the figs are picked several days before maturity, the insects, unless artificial heat is applied, develop at so slow a rate that only about 20 per cent of them emerge during the four or five days when sterilization is effective. (2) The dipped figs must be destroyed within five days of treatment.

To obviate these drawbacks a new plan was devised (9). It has previously been thought that the blastophaga insect is very short-lived after it emerges from the gall (4). The writer found by experiments that emerged insects placed in glass vials or other containers and stored in the dark at temperatures ranging from 35° to 50° F. would remain alive for eight days and at the end of that time were able to enter and properly fertilize caprifigs. The longevity of the insects, together with the facts that they are positively phototropic and in their rate of emergence respond readily to heat, made it possible to augment the dipping method by insectary practices.

Mamme figs were picked about four weeks before maturity and stored at about 60° F. They were then treated by the dipping method and placed in a specially constructed incubator (Fig. 2) at about 85° F. At this temperature it was found that from 60 to 80 per cent of the insects would emerge within 24 hours. The insects were made to emerge into sterilized vials. When about 500 insects had entered a vial it was removed from the incubator, corked, and cooled to about 45° F. Shipments of such vials by mail and express to various parts of the state indicated that, if properly handled, the insects were not adversely affected by transportation, as upon liberation in capri trees they entered and properly fertilized the caprifigs.

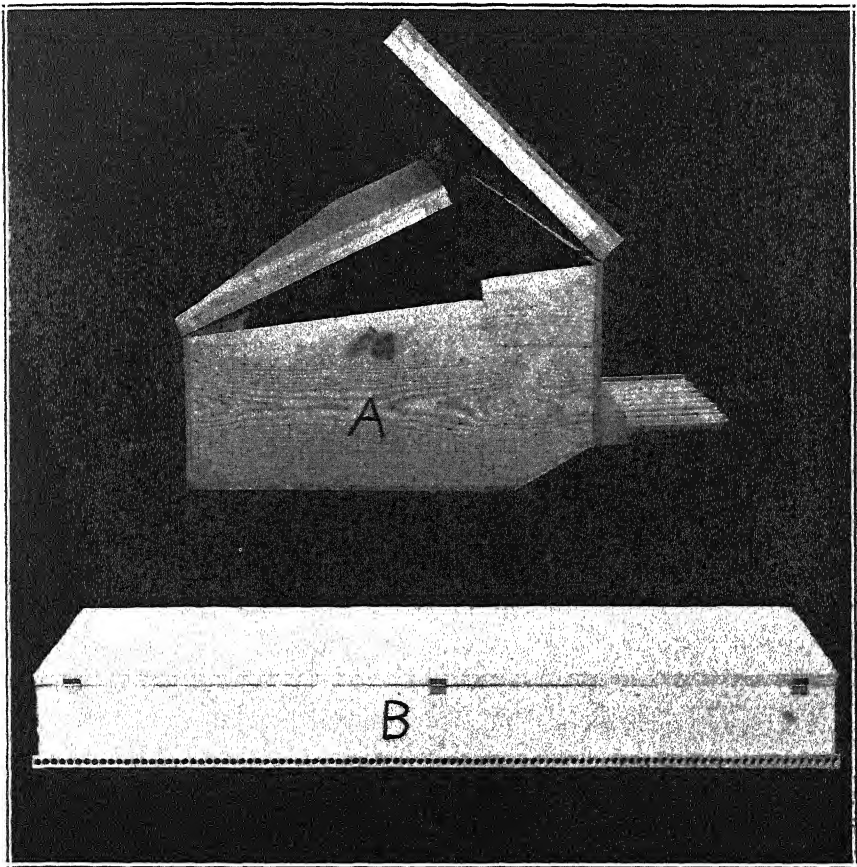


FIG. 2. Special incubator. A. End view, with glass vials in place. B. Front view. The only light entering the incubator comes through the holes in which the glass vials are placed. The dimensions of the incubator are approximately 1 ft. x 3 ft. x 10 ft. The bottom is of sheet metal; the front, rear and top of celotex; and the ends of wood.

By this plan it should be possible to remove all the mamme figs in the state and ship them to an insectary in a place where no blastophaga could escape to any caprifig trees. There the figs would be stored and treated by the dipping method. By use of special equipment and by application of heat the insects at the proper time would be made to emerge into sterile vials in which they might be shipped to any part of the state and placed in trees to caprify the profichi crop. This method would make it unnecessary to remove any treated figs from the trees, furnish large numbers of clean blastophaga, and make it possible to clean up the whole territory in one season.

SUMMARY

1. A rot (endosepsis) of the fruit of caprifiged figs in California has assumed alarming proportions in recent years.

2. The causal organism, *Fusarium moniliforme* Sheld. var. *fici* Cald. grows and develops in the various crops of caprifigs and is transmitted from them to the calimyrnas by the caprifying insect *Blastophaga psenes* L.

3. The rapid increase and spread of the disease is attributed to the practice of inter-sectional distribution of caprifigs, to the prolific nature of the insect and its ability to fly long distances.

4. Laboratory and field experiments indicate that the causal organism may be eliminated from caprifigs either by injecting a fungicide (2 per cent Semesan) into young capri (mamme) or by cutting mature ones into halves and dipping them for 15 minutes in the fungicide.

5. The dipping method may be made adaptable to large-scale use by application of insectary methods.

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DEVELOPMENT OF STORAGE DISEASES IN SUGAR BEETS RESULTING FROM HOOK INJURY

C. M. TOMPKINS AND S. B. NUCKOLS¹

The sugar beet (*Beta vulgaris* L.) is especially susceptible to fungous invasion immediately following any form of bruising or mechanical injury at harvest time, because of the comparatively thin layer of cork cells constituting the periderm. Injuries to the root may occur in a number of ways, but this paper is concerned particularly with wounds in the beet occasioned by the use of the so-called hooked beet knife, with subsequent development of decayed areas due to fungous attack.

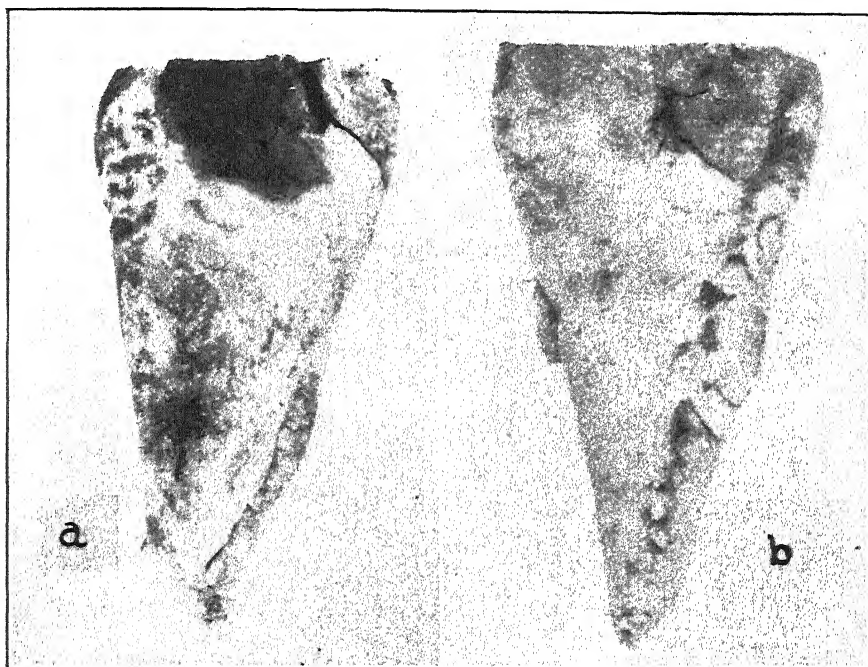


FIG. 1. Sugar-beet roots, (a) showing hook injury and splitting of tissues with subsequent development of decay; (b) showing hook injury and subsequent development of decay.

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In the United States, topping machinery has not come into commercial use and practically all sugar beets are topped by hand. A straight beet knife is used in Colorado, Nebraska, Montana, Michigan, and Ohio, the use of which necessitates raising each individual beet by hand from the ground or nearby pile. This method precludes the possibility of injury to the beet root, insofar as topping practice is concerned, and is to be highly recommended. In the states on the Pacific slope, including Utah, Idaho, Washington, and California, a hooked knife is used. This knife is approximately the same size as the straight beet knife but in addition is equipped with a sharp-pointed, curved hook at the extremity of the knife blade. The hook is 7 cm. long, with a mean diameter of 7 mm., and tapers down to 2 mm. at the tip. The tapered portion of the hook occupies a length of not more than 10 mm. In order to raise a beet from the ground, the laborer, with a quick stroke of the knife, drives the hook into the root, usually on the lateral side of the crown. Observations and examinations have shown that invariably the hook is forced into the tissues sufficiently far to include its greatest diameter. When the hook is withdrawn, a wound with a mean diameter of 7 mm. has been made (Fig. 1). Often this wound is larger than 7 mm., particularly if difficulty is experienced by the laborer in withdrawing the hook from the beet when once it has been raised. Sometimes two or three wounds will be made in the root by the hook as the laborer attempts to hook the beet. Severe splitting of the tissues is not an uncommon occurrence. Often a very ragged edge results which is ideally suited for incubation of fungous spores.

Since factory capacities are such that all beets harvested in any single day can not be processed immediately, many tons of roots must be stored



FIG. 2. Cross and longitudinal sections through the center of the injured portions of diseased sugar beets, showing the effect of hooked-knife injury.

for various periods, the average of which is approximately 40 days. Parasitic fungi, under favorable conditions of air temperature and humidity, are able to cause decay in wounded beets to the extent of from 15 to 25 per cent of the total weight of any individual root in a relatively short time. Frequently one-quarter to one-half of the root will become involved (Fig. 2). In commercial storage piles where beets are held for from 25 to 40 days or longer, it is estimated that a loss of not less than 15 per cent is sustained. This includes both weight and sugar loss. Root injury such as described above constitutes a source of tremendous but avoidable loss to sugar companies.

Numerous isolations have been made from diseased beets, disclosing the fact that *Phoma betae* is the principal wound parasite responsible for decomposition of the tissues. Several species of *Fusarium* also are parasitic. These organisms are unable, apparently, to parasitize the beet root in the absence of wounds.

In order to prevent future losses in storage from this source, it is recommended that the use of the hooked knife be dispensed with and the Colorado system of harvesting substituted.

OFFICE OF SUGAR PLANTS, BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
IN COOPERATION WITH
UTAH AGRICULTURAL EXPERIMENT STATION,
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PHYTOPATHOLOGICAL NOTES

Observations on the discharge of ascospores of Venturia inaequalis in Maryland.—Prior to 1925 it was customary for Maryland fruit growers to make their first spray application for the control of apple scab during the pink-bud stage of the tree. The failure of many of these growers to control scab successfully led to studies on the time of first ascospore discharge in the spring. The results of these studies during the past four years are as follows.

In the spring of 1925 fallen apple leaves containing scab lesions were collected in an orchard in the southeastern part of Maryland. On March 2 they were placed in moist chambers, one lot being kept in a greenhouse and another lot in an insectary outside of the building. The leaves were moistened at intervals of one to two days. The leaves in each chamber were covered with a piece of large-mesh wire screen, like that used on basement windows. Microscope slides were greased with vaseline and placed on the wire screen over the leaves. These slides were replaced daily and examined with a microscope. On March 27 the first ascospores were found on slides from the greenhouse, and on March 28 the first spores were found on the slides from the insectary. The average temperature in the greenhouse during the period March 2 to 27 was approximately 60° F., and that in the insectary during the same period was about 45° F. For several days after March 28 ascospores were found on both sets of slides. It was thus evident that ascospore development was practically as rapid at an average temperature of 45° as it was at 60° F. from March 2 to 27.

In the spring of 1926, fallen apple leaves containing scab lesions were collected in five orchards located in different parts of the state. These leaves were placed on burlap sacks and held in place with large-mesh wire screening, which was spiked to the ground. Two lots were placed in each orchard. One lot was left under natural conditions and the other sprinkled daily with tap water. Slides greased with vaseline were placed over the leaves and examined daily. The first dates when ascospores were found are indicated in table 1.

In all cases there were several showers and rains between the time observations were begun and the time the first ascospores were found on the slides. At Ridgely there were several more showers than in the other orchards where the work was conducted. In no cases did the number of rains equal the number of times the leaves were moistened. In all orchards, except at Ridgely, there were several rains between the time of finding the first ascospores on the slides over the moistened leaves and the finding of the

TABLE 1.—*The maturation of ascospores of Venturia inaequalis in Maryland in the spring of 1926*

Place	Date of collection	Date on which ascospores were first found ^a	
		Over moistened leaves	Over leaves under natural conditions
Ridgely	March 19	April 28	April 29
Snow Hill	March 24	April 15	May 3
Whiteford	April 4	April 21	May 15
College Park	March 28	April 23	May 16
Newburg	April 3	April 12	April 18

^a Ascospores caught on glass slides exposed above the infected apple leaves.

first ascospores on slides over the leaves kept under natural conditions, indicating that ascospores on the latter were not ready to discharge so soon as those on the moistened leaves. In all cases there was rain on the day the first ascospores were found on the slides over leaves kept under natural conditions. It thus appears that up to a certain point the more frequently the apple leaves are moistened the earlier the ascospores will mature.

The season of 1926 was unfavorable for the development of apple scab in Maryland, and scab lesions were found on only a very small number of apples leaves. When the studies of the perithecia were resumed in the spring of 1927, it was almost impossible to find any fallen leaves showing scab lesions. However, fruiting bodies which could not be distinguished from the perithecia of *Venturia inaequalis* (Cke.) Wint. were found on practically every fallen leaf which was examined. Many of the leaves were practically covered with them, and they were apparently as prevalent on leaves from sprayed orchards as they were on leaves from unsprayed orchards. When these bodies matured they developed asci and ascospores that were identical in measurements and appearance with those developed in perithecia on leaves with conspicuous scab lesions. In the spring of 1928 the perithecia of *V. inaequalis* (Cke.) Wint. were again found on practically all of the fallen leaves examined from sprayed and unsprayed orchards in all parts of Maryland. No visible scab lesions could be found on most of these leaves, although they bore abundant perithecia.

Perithecia were thickly scattered over the entire surface of some of the leaves, including the midrib and the petiole, while on others they were found only in scattered groups. Perithecia occurred on either the upper or lower sides of the leaves, being most abundant on the surfaces exposed to light. They were found abundantly on all varieties examined, including Winesap, Stayman Winesap, Northwestern Greening, Yellow Transparent, Delicious, Ben Davis, and York Imperial.

In 1927 the first ascospore discharge occurred at widely different stages in the development of the trees in different sections of the state, varying from the so-called "pre-pink" stage in the southeast to the calyx stage in the extreme western part. In 1928, in the extreme southeastern part of the state, the first discharge occurred while the trees were still dormant.—R. A. JEHL and H. A. HUNTER, University of Maryland, College Park, Md.

Bacterial red-stripe disease of sugar cane in countries of the Pacific.—Bacterial red-stripe disease of sugar cane was first recorded in 1924 from the Hawaiian Islands,^{1,2} where it was of economic importance only upon Tip varieties of cane, which are gradually being supplanted by higher-yielding varieties.

There is a bacterial red-streak disease of sugar cane in Queensland, Australia, reported in 1926 by W. Cottrell-Dormer,³ which in photographs appears identical with the bacterial red-stripe disease found in Hawaii.

Edgerton and Christopher⁴ recorded the presence of the same disease in Louisiana three years later on the variety D. 74, a variety which also is gradually being supplanted by higher-yielding canes.

In a recent report by Bolle,⁵ red-stripe disease of sugar cane has been recorded from Java. The cane affected was the Java variety, P.O.J. 2722.

In the Philippines, specimens of a disease were collected by one of the writers of this note (Pierce), and on the arrival of Lee in the Philippines it was identified as identical with the red-stripe disease of sugar cane previously recorded in the Hawaiian Islands. The cane affected was the Java variety, P.O.J. 2883.

These findings point to the rather general distribution of the disease in countries of the Pacific, although its presence is not recognized until susceptible varieties are grown. The disease is of very little commercial importance at the present time, because most of the standard sugar cane varieties are highly resistant to it.—H. ATHERTON LEE and W. DWIGHT PIERCE.

¹ LEE, H. ATHERTON and W. C. JENNINGS. Bacterial red-stripe disease of Tip canes. Hawaiian Sugar Planters' Assoc., Experiment Station Circular 42. 1924.

² LEE, H. ATHERTON, J. P. MARTIN, HELEN A. PURDY, CLYDE C. BARNUM, and D. M. WELLER. Red-stripe disease studies. Hawaiian Sugar Planters' Assoc., Experiment Station Publication. September, 1925.

³ COTTRELL-DORMER, W. Notes and observations on the red streak associated with Queensland top-rot disease. Queensland Agr. Jour. 25: 406-414. 1926.

⁴ EDGERTON, C. W., and W. H. CHRISTOPHER. Red-stripe disease of sugar cane. The Planter and Sugar Manufacturer, 79⁴: 63. 1927.

⁵ BOLLE, P. C. Verdere Onderzoekingen over Pokkahboeng en Toprot. Korte Mededeelingen van het Proefstation voor de Java-Suikerindustrie, Jaargang 1928, No. 4. Abstract in Rev. Appl. Mycol. 7: 537. 1928.

Cladosporium Diseases.—A recent article¹ by Bensaude and Keitt, "Comparative studies of certain *Cladosporium* diseases of stone fruits," interested me somewhat because many years ago I published a good many notes on *Cladosporium* on several of our stone fruits. Bensaude and Keitt evidently overlooked these early papers.

I first came in contact with the fungus on plum and cherry in 1889. Somewhat later I discovered a scab on the American plum, which I thought might be caused by a new species of *Cladosporium*; but, after a careful study of the mycelium and spores, I came to the conclusion that the fungus was identical with the one on peach. The *Cladosporium* on peach had been studied by Erwin F. Smith.²

In the Ottawa Naturalist for November, 1892, is an article entitled "A destructive disease affecting native plums," written by John Craig. Most of the article was written by myself and should have been in quotation marks.

I made numerous references to the *Cladosporium* on stone fruits in my notes on diseases of plants at Ames, published in the Proceedings of the Iowa Academy of Science 2: 207. 1894. I also mentioned it in a paper on "New fungous diseases of Iowa," published in the Journal of Mycology, 7: 95-103. 1891. In that paper I referred to its having been seen previously by H. Osborn and to the careful work of Van Thümen, Erwin F. Smith, and B. T. Galloway. Those specimens from Ames were submitted to J. B. Ellis, who thought that the fungus might be a distinct species, but after careful study I concluded that the fungus was *Cladosporium carpophilum*.

I have referred to this *Cladosporium* on stone fruits and the importance of the disease to horticulture in many subsequent papers (See reports of Iowa State Horticultural Society, Iowa Academy of Science, Society for Promotion of Agricultural Science, Iowa Agricultural Experiment Station, and Iowa Weather and Crop Service).—L. H. PAMMEL, Iowa State College of Agriculture, Ames, Iowa.

¹ Bensaude, M., and G. W. Keitt. Comparative studies of certain *Cladosporium* diseases of stone fruits. *Phytopath.* 18: 313-330. 1928.

² Smith, Erwin F. Spotting on peaches. *Jour. Mycol.* 5: 32-33. 1889.

REPORT OF THE TWELFTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The meetings were held in conjunction with those of the Pacific Division of the American Association for the Advancement of Science and Affiliated Societies at Pomona College, Claremont, California, June 13-16, 1928, and were called to order by vice-president A. G. Milbrath.

A business meeting was held and the following officers elected to serve the society the next two years:

PresidentJ. W. HOTSON, University of Washington, Seattle.
Vice-PresidentEUBANKS CARSONER, University of California, Citrus Experiment Station, Riverside.
Secretary-TreasurerB. A. RUDOLPH, University of California, Deciduous Fruit Station, San Jose.
CouncilorJ. T. BARRETT, University of California, Berkeley.

A motion was introduced to appoint a committee to draw up a suitable amendment to the constitution which would make it possible for the Society by vote of its members present at annual meetings to express to the author of the most outstanding piece of research presented at the meetings its admiration and approval in a material way in the form of a book on plant pathology or kindred subject, a bit of apparatus, or even a sum of money. The motion lost by one vote.

A suggestion that the Society undertake the preparation of a highly illustrated manual of plant diseases found in the territory embraced by the Pacific Division and suitable as a ready reference book for field use rather than for technical laboratory work met with general favor. Owing to the lateness of the hour no action was taken.

A motion to appoint a committee to draw up an amendment to the constitution providing for the automatic elimination of members not in financial good standing in the Society was passed. This amendment must be submitted to the members of the Society and accepted by a majority vote before it can be introduced into the constitution.

Twenty papers were presented, the titles and abstracts of which follow.

B. A. RUDOLPH, *Secretary-Treasurer*.

ABSTRACTS

Rusts of the Pacific Northwest.—J. W. HOTSON.

The article consists of a somewhat elaborate key to all the rusts reported from Washington, Oregon, Idaho, and Montana. Since individual rusts in general are restricted quite closely to a few hosts, an attempt has been made to make a key on the basis of these hosts. All the rusts of the region are divided according to the host-family, and then subdivided under the genus of the host. The individual characteristics of the rusts are used to separate those occurring on the same genus.

Armillaria mellea in mines and wells.—J. W. HOTSON.

Armillaria mellea has been found abundantly on wooden supports in several coal mines in Washington. It has also been found on wooden curbing in wells. In both locations rhizomorphs were abundantly produced, hanging down in great festoons from the timbers in the mines, and floating on the water in the wells. Although the cultures obtained directly from a well have not as yet produced sporophores, the form and structure of the rhizomorphs produced are identical with similar cultures made from the spores of *Armillaria mellea*, leaving little doubt as to the identity of the fungus.

An Aspergillus attacking mealybugs in insectaries in southern California.—A. M. BOYCE and H. S. FAWCETT.

A species of *Aspergillus* appears to be a potential pest in insectaries where mealybugs are propagated for their value in rearing beneficial insects. Notes are given on the culture and parasitism of this fungus.

Inheritance of resistance to blast in oats.—W. W. MACKIE.

Blast in oats causes sterile, blanched, and reduced spikelets, resulting in severe crop losses. Many attempts to isolate a causative organism have failed. Explanations for its cause on the basis of malnutrition have not satisfactorily accounted for the disease. Many years of observations with over 300 varieties of oats indicate that varietal resistance is approximately stable. A cross between Kanota, an approximately immune variety, and Richland, a susceptible variety, gave all susceptible plants in the F_1 generation. In the F_2 generation the plants were observed to resist blast as follows:

Plants highly susceptible.....	108
Plants moderately susceptible.....	356
Plants immune or with only a trace.....	178

These numbers indicate a very close approximation to a 1:2:1 Mendelian ratio, indicating a single factor for blast resistance.

Phytophthora in relation to crown rot of walnut.—J. T. BARRETT.

The disease of English walnut trees known as crown rot has been under observation about five years. The lesions are at first confined to the crown and to the basal part of the roots of the California black walnut species used as stocks. The southern black species, *Juglans californica*, seems more susceptible than the northern species, *J. hindsii*. The white or English root is very resistant. The crown rot lesions may extend onto the English walnut trunk following girdling of the crown and a weakened condition of the tree. It is from these trunk lesions that *Phytophthora cactorum* has been repeatedly

isolated. The disease has been produced by inoculation with this organism into both the black and English species.

The inorganic nutrition of the fungi. I. The relation of calcium and boron to growth and spore formation.—A. R. DAVIS, R. H. MARLOTH, and C. J. BISHOP.

Information on the inorganic salt requirements of the fungi is meager, particularly so with respect to elements which may be needed in small amounts. C. P. chemicals employed in making up nutrient media usually contain as contaminations sufficient amounts of many elements to supply the needs of the organism. In the present experiment the salts were carefully recrystallized and purified. In the case of *Aspergillus niger* and *Penicillium italicum* 50 cultures were grown on media made up of purified and unpurified salts with and without calcium, and the data treated statistically. With both these organisms calcium was shown definitely to increase yield over that obtained in media devoid of this element, and spore formation was dependent upon the presence of the ion. When *Dothiorella sp.* was employed as a test organism, the absence of boron in the medium decreased yields about one half, and the addition of one and one half part to a million restored yield to 90 per cent of that obtained with the unpurified salt. The evidence indicates that both calcium and boron are to be regarded as essential and not as stimulants in the older sense.

The effect of mixed inoculations of certain citrus fruit-rotting organisms.—G. SAVASTANO and H. S. FAWCETT.

Inoculations were made in uniform wounds of orange and lemon fruits with mixed suspensions of spores from pure cultures of different species of fungi and the effect of these mixtures compared with that of each fungus inoculated singly. Mixtures of two or more of the following Citrus-fruit-rotting fungi were used: *Penicillium italicum*, *P. digitatum*, *Aspergillus niger*, *Trichoderma lignorum*, *Oospora citri-aurantii*, *Pythiacystis citrophthora*, *Botrytis cinerea*, *Sclerotinia libertiana*, *Alternaria citri*, *Phomopsis californica*, *Diplodia natalensis*, and *Dothiorella ribis*. Different lots of fruit were placed at maintained temperatures from 3° to 33° C. Some of the most important features brought out by this investigation were (1) the selective effect of temperature in enabling one organism in a mixture to dominate over the others in producing decay; (2) the depressing or accelerating effect of given mixtures on the rate of decay as compared with that produced by the most rapidly advancing organism of the mixture when used alone; (3) the influence of given mixtures of organisms on the type of decay, its color and consistency, and (4) the differences in temperature range, and in optimum, maximum and minimum temperatures of the various organisms for rate of decay during a given time. The presence of *Oospora citri-aurantii* in certain mixtures, especially with *Penicillium digitatum*, caused marked increase in some cases, the rate of decay being more than the sum of the rates due to the two organisms when used separately. The mixture of the two *Penicilliums* showed a depressing effect at most temperatures, except the highest and lowest temperatures used. The presence of *Botrytis cinerea* in certain mixtures also had a depressing effect on rapidity of decay as compared to that of the most rapidly advancing organism of the mixture. Some of the mixtures produced characteristics of color and consistency of decaying tissue that helped to elucidate effects not previously well understood in the orchards and packing-houses.

The brown blight disease of lettuce.—IVAN C. JAGGER.

Brown blight is a new disease of lettuce, which has caused increasing losses in the Imperial Valley of California. It appeared soon after the beginning of lettuce culture

there about 12 years ago. More recently it has been found in Arizona and in several other lettuce sections of California. Plants are attacked in all stages of growth from three or four leaf seedlings to maturity. Young plants become yellowish, more or less mottled, much stunted, and gradually die, while on plants that are not attacked until they have attained some size there are, in addition, conspicuous dead brown streaks and blotches on the leaves. The disease is soil borne. It may occur in the first crop of lettuce to a limited extent and increases rapidly from year to year with constant cropping. No appreciable control has been obtained by growing non-susceptible crops on diseased soil for three or four years. The cause has not been definitely determined but a parasitic organism in the roots is indicated. The fungus *Asterocystis radialis* de Wildeman has been found rather constantly associated, but definite proof of causal or secondary relation is as yet lacking. Highly resistant strains of the New York variety ("Iceberg lettuce") have been developed and are being extensively grown in Imperial Valley. At present "Imperial No. 2" strain is the most extensively used with a considerable acreage of "Imperial No. 3" and a few trial fields of the more recently developed "Imperial No. 6."

The occurrence of Peronospora sparsa Berk. on hot-house roses in southern California.—O. A. PLUNKETT.

Altho *Peronospora sparsa* seems to be common and frequently destructive in Europe, it has received little attention in the United States. It appeared in several greenhouses near the coast of southern California in the early fall of 1927 with the advent of cool foggy nights and warm days. The fungus causes a spotting of young foliage followed by a dropping of the leaflets. Under humid conditions abundant conidiophores and conidia are produced in the spots on the undersides of the leaves. The disease is not serious enough to kill the plants but delays the time of blooming two or three weeks, which keeps the roses off the Christmas market, thus causing considerable loss. The disease seems to be associated with poor air drainage, an excess of humidity, a high temperature during the day and a low temperature at night. It has been controlled successfully by proper ventilation, regulation of the humidity and temperature, and by thorough and frequent spraying with bordeaux 5-5-50.

The development of tomato yellows under different light conditions.—MICHAEL SHAPOVALOV and F. S. BEECHER.

Experiments with artificial shading of tomato plants, which tended to lower the rate of evaporation, reduced the amount of yellows (formerly known as western yellow blight). While some of this reduction under field conditions may have been due to the protection of the plants from beet leafhoppers, instrumental in spreading the infection, it was also attained in cases when tomatoes were artificially inoculated and then shaded. Inoculation tests conducted simultaneously under different habitats show very definitely that light is of primary importance in the progress of tomato yellows and correlates very closely with the rate of evaporation. However, indications are that low relative humidity also favors the disease to a certain extent. The total intensity of light during these experiments was measured at various times by the uranyl acetate-oxalic acid method. The effect of reduced light was four-fold: (1) the incubation period in the host was prolonged; (2) the plant growth was accelerated; (3) the disease symptoms took on a milder form; and (4) the percentage of yellows was lower.

The influence of sodium chloride in alkali soils on the susceptibility of Pima cotton to angular leaf-spot caused by Phytomonas malvaceae.—J. G. BROWN.

Experiments carried on by the writer since 1921 in the field, screened garden, and laboratory show that Pima cotton grown on soil containing 0.2 to 0.4 per cent of sodium chloride is resistant to angular leaf-spot. When the concentration of sodium chloride is near the limit of tolerance of cotton (0.4 per cent), Pima cotton is practically immune to the disease. Sodium carbonate in the soil shows no protective effect. The resistance observed appears to bear no relation to osmotic concentration. It is probably due to the accumulation of Cl in the parenchyma where it can be detected by microchemical methods. The series of experiments is of interest in connection with the growing of cotton on alkali soils.

Further studies on attenuation of the virus of sugar beet curly-top.—EUBANKS CARSONER and C. F. LACKEY.

In connection with the study of resistance to the curly-top disease, resistant sugar beets have been infected by means of viruliferous leafhoppers and the virus then transferred from these diseased plants to healthy susceptible sugar beets by the use of non-viruliferous leafhoppers. The tests reveal that under certain conditions, which are as yet not well defined, the passage of the curly-top virus through resistant beets results in a marked attenuation of the virus. The degree of attenuation varies to such an extent that when small plants of a susceptible strain are inoculated with the attenuated virus the resulting symptoms range from the severity characteristic of the disease when due to the active or unattenuated virus to the very mild form produced by the virus after it has been passed through *Chenopodium murale*. The variation in degree of attenuation appears to correspond directly to the severity of the symptoms on the infected resistant plant used as the source of the attenuated virus. Attenuation of the virus by resistant sugar beets has been demonstrated for three different strains. The evidence thus far available indicates that neither prolonged development of the attenuated virus in a susceptible plant nor repeated passage of it through susceptible plants restores its virulence.

Measurements of total daily sunlight intensity with reference to the ecology of plant diseases.—F. S. BEECHER.

Need for a readily portable, inexpensive, and reasonably reliable method for measuring the cumulative effect of sunlight in the study of plant diseases led to the adoption of Dr. R. F. Bacon's chemical photometer method, using uranyl acetate and oxalic acid.

By this method the percentage of total sunlight available to plants under various conditions appeared to be, on the average, about as follows: Open frame 87 per cent, frame with thin single glass 70 per cent, with double glass 60 per cent, thin muslin 47 per cent, medium weight muslin 40 per cent, heavy muslin 8 per cent, shady portion of greenhouse 15 to 20 per cent, central greenhouse 30 to 35 per cent, and sunny portion 35 to 45 per cent. The very small effect under both the Uviarc quartz lamp and the 500-watt Mazda showed how inadequate they are for plant illumination, as compared with sunlight, as well as possibly suggesting that the photochemical effect may be produced by a fairly wide range of the spectrum, rather than being confined to the violet and ultra-violet portion.

As a method for use in the field and greenhouse this chemical photometer has seemed quite satisfactory.

A study of citrus blast and some allied organisms.—C. O. SMITH.

Study has shown a great similarity in the pathogenicity and cultural characteristics of *Bacterium citriputeale* from avocado and Citrus, *Bact. syringae* from lilac in California, and *Bact. cerasus* from apricot. The cultural characteristics are in accord with the description¹ of the lilac organism. The following are some of the hosts that were successfully inoculated by puncture with each of the species mentioned: fruit of *Prunus armeniaca*; twigs of *P. armeniaca* but slightly infected except with *Bact. cerasus* where a lesion about 20 millimeters resulted; fruit and twigs of avocado; twigs of *Chalcas exotica*, one of the Citrus relatives; twigs and leaves of *Coprosma baueri* (a susceptible host); twigs of *Jasminum primulinum*, *Frazinus floribunda* (S. P. I. 47687); fruit of lemons; orange twigs, lesions small, but typical; fruit of tomato; lilac leaves and twigs; leaves and twigs of *Populus sp.*; fruit and twigs of pears; fruit and twigs of apple. Reisolations and reinoculations were made to complete the rule of proof. From the study thus far given the results seem to indicate that the three species are closely related culturally and pathogenically. They may be found to belong to the same species, but more study is necessary to determine this, and no change is suggested in the nomenclature at present.

Steam sterilization of coniferous seed-beds.—T. C. SCHEFFER.

Experimental work done during the past season to determine the efficacy of steam sterilization by the inverted pan method when applied to coniferous seed-bed soil has indicated excellent possibilities for its use. Three western genera of conifers, Douglas Fir, Sitka Spruce and Western Yellow Pine, were used in the investigation. In the case of each genus, seedling counts made in the fall showed the plants in the control beds to be outnumbered three to four by the plants in the beds which had been steamed in the spring. Root systems of plants in the steamed soil also showed a greater development. Weeding costs for the steamed beds were reduced to but one-sixth of those of the control beds. One-hour and two-hour steaming periods were used, employing low pressure steam, with results indicating that these were in excess of the time actually needed to sterilize sufficiently for fungi. The damping-off organisms involved seemed to be *Fusarium sp.* and *Phoma sp.*

Eocronartium musicola fr. in a new locality and on a new host.—KEITH O'LEARY.

In July of 1926 *Eocronartium musicola* was found on the moss *Leucolepis acanthoneura* (Schwaegr.) Lindb. at Sedro-Wooley, Washington. *Leucolepis* adds a third family, Mniaceae, to the list of hosts, the other families being Leskaceae and Hypnaceae. This is apparently the first time that this interesting fungus has been found in the west. Several attempts were made to culture the fungus on the ordinary laboratory media but all failed. Some field inoculations were attempted by dusting the sporophores over the moss, but this work, too, gave negative results.

Studies of Texas root rot in Arizona.—R. B. STREETS.

Texas root rot caused by *Phymatotrichum (Ozonium) omnivorum* causes the greatest economic loss of any plant disease occurring in Arizona. The life history of the fungus and control measures have been studied the last two years. Efforts to germinate the conidia which are often produced in great abundance have been unsuccessful, although a

¹ BRYAN, MARY K. Lilac blight in the United States. Jour. Agr. Res. 36: 225-235. 1928.

large number of media have been tried. The fungus is polymorphic, advancing through the soil by means of rhizomorphs composed of a large central hypha surrounded by a sheath of finer mycelium bearing the characteristic slender cruciform-branched appendages. The primary infection on cotton and alfalfa is usually on the taproot from 6 to 12 inches below the soil surface. Primary invasion is accomplished by a combined wedging action of the hyphae between the cortical cells and the penetration and filling of the adjacent cells with hyphae. A small recently formed lesion shows scores of points where the fungus has penetrated the cortex. Special technique is necessary to isolate the organism and inoculate healthy plants. One hundred selections from very severely infested fields of Pima cotton and a like number of Acala cotton have been grown for two years on heavily infested soil. Certain of the surviving progenies show promise of a satisfactory degree of resistance. Soil sterilization with chemicals has proved effective when the infected spot is small and cost of treatment is not the limiting factor. Formaldehyde, organic mercury salts, carbon bisulphide, and sulphuric acid were the most effective chemicals used.

Inhibition of enzymatic action as a possible factor in the resistance of plants to disease.—

L. J. KLOTZ.

During the course of an investigation which seeks to throw some light on possible bases for the resistance of sour orange (*Citrus aurantium* L.) and for the susceptibility of lemon (*Citrus limonia* Osbeck) to the bark diseases gummosis and deortiosis, caused by *Pythiacystis*, it has been found that the trunk bark of sour orange has a much greater inhibitory or paralyzing influence on the action of certain enzymes found in the dried mycelial powder of the casual fungi than does the trunk bark of lemon. This suggests the possibility that resistance to the invasion of the pathogens may be due to the inhibition of one or more of the enzymes of the fungi by some cellular product of the host, and that a sufficient decrease in this paralyzing power might permit the hyphae to progress rapidly, as they do in the bark of the susceptible lemon, and successfully paralyze the host.

Similar tests with other varieties of Citrus have given results approximating the degree of susceptibility and resistance to *Pythiacystis* gummosis as indicated by lesions resulting from pure culture inoculations under field conditions.

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PHYSIOLOGIC SPECIALIZATION IN SOME CEREAL SMUTS¹

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INTRODUCTION

The discovery of the phenomenon of physiologic specialization in the fungi is undoubtedly one of the most important developments in plant pathology. It was first suggested by Schroeter (43), in 1879, and since that time physiologic specialization has been found to be of very wide occurrence among plant pathogens. Reed (33), in 1918, summarized in detail the information on physiologic specialization in the fungi which was available at that time. From 1879 to 1918 classic work was done on physiologic specialization in the rusts and powdery mildews, but investigations on the phenomenon in the smut fungi seem to have been neglected. Kniep (28), in 1919, was the first to show that physiologic specialization does occur in the smut fungi. He noticed differences in the appearance of the sporidial cultures of *Ustilago violacea* (Pers.) Fuckel from different host plants. Zillig (51), in 1921, then demonstrated that there were physiologic forms of this fungus that could be differentiated on the basis of their ability to infect certain members of the Caryophyllaceae and not others. This work was later confirmed by Liro (32) and Bauch (4).

The earlier investigators distinguished physiologic forms only by pathogenicity. Now, however, physiologically distinct entities within a species can be distinguished on the basis of cultural characters (6, 7, 9), physico-chemical reactions (13, 21, 24, 27), and, in some cases, by differences in morphology (1, 31). Thus, according to Stakman (44), there are four general methods for recognizing physiologic forms: cultural characters, physico-chemical relations, morphology (to a limited extent), and pathogenicity. The study of cultural and physico-chemical differences within a

¹ Published with the approval of the director as Paper No. 797 of the Journal Series of the Minnesota Agricultural Experiment Station.

² The writer takes pleasure in acknowledging his indebtedness to Dr. E. C. Stakman, at whose suggestion the research was undertaken, for the encouragement and helpful criticism he gave during the investigation. The writer also wishes to express his appreciation to Dr. J. J. Christensen for many helpful suggestions offered during the progress of the investigation.

species of a fungus is of importance since they may serve as the first indication of pathogenic differences. In fact, as has been pointed out, physiologic specialization in the smut fungi was first discovered by finding differences in cultural characteristics of *U. violacea*. Christensen and Stakman (9) differentiated 15 physiologic forms of *Ustilago zae* (Beck.) Ung. on the basis of cultural characteristics. At least 7, and possibly 8, of these forms were then differentiated on the basis of their parasitic behavior on 10 selfed lines of corn. Broadfoot (5) distinguished 9 physiologic forms of *Fusarium lini* Bolley by differences in cultural characters. All of these forms were then differentiated by their parasitism on four varieties of flax. Similar results have been obtained by Christensen (8) with *Helminthosporium*



FIG. 1. Pure culture of *Ustilago avenae* obtained from chlamydospores which had been washed previously in a 1 per cent solution of copper sulphate for a period of 27 hours.

sativum P. K. and B. and by Ezekiel (14) with *Sclerotinia americana* (Wormald) Nor. and Ezek. Letcher and Willaman (30) found that the physiologic forms of *F. lini*, which were originally recognized by cultural characters and pathogenicity, differed also in their ability to produce alcohol from sugar.

In the production of disease-resistant varieties of crop plants the study of physiologic specialization in the fungi is of great importance. Varieties which are immune or resistant to certain pathogenes in one locality may be entirely susceptible when grown in another geographical area. In a study of physiologic forms of oat smuts, Reed (35) found striking differences in the reaction of varieties of oats to smuts collected in different geographical areas. *Avena brevis* Roth has always been highly resistant to *Ustilago levis* (Kellerm. and Swingle) Magn. when inoculated with the smut collected in Missouri, but when inoculated with the smut from Wales 100 per cent of the plants became infected. A virulent form was found in the United States, however, which did not occur in Wales. One hundred per cent of the plants of Canadian, a variety of *Avena sativa* L., became infected when inoculated with *U. levis* from Missouri, but the Welsh smut caused no infection whatever. Sampson (41) obtained results similar to those obtained by Reed with physiologic forms of smuts collected in Missouri and in Wales. Similar results have been obtained by Faris (16) on the reaction of varieties of barley to physiologic forms of *Ustilago hordei* (Pers.) K. and S. Tisdale and Griffiths (49) found differences in the reaction of wheat varieties to physiologic forms of *U. tritici* (Pers.) Rost.

The investigations reported in the present paper include a study of the cultural differences and physico-chemical relations between physiologic forms of some of the cereal smut fungi. The investigations also include a study of varietal resistance to smut in wheat, oats, and barley with reference to the pathogenicity of physiologic forms.

SPECIFIC OBJECTS OF THE INVESTIGATION

The objects of the present investigation were:

1. To ascertain the number and distribution of physiologic forms of *Ustilago tritici*, *U. nuda*, *U. hordei*, *U. avenae*, *U. levis*, *Tilletia levis*, and *T. tritici*.
2. To ascertain the possible differences in cultural characteristics and the effect of temperature on the growth of physiologic forms of *U. tritici*, *U. nuda*, *U. hordei*, *U. avenae*, and *U. levis*.
3. To ascertain the possible differences in pathogenicity of *T. levis*, *T. tritici*, *U. hordei*, *U. levis*, and *U. avenae*.
4. To determine the varietal resistance of wheats to *T. levis*, and of oats to *U. levis* and *U. avenae*.

CULTURAL DIFFERENCES BETWEEN PHYSIOLOGIC FORMS

During 1925 and 1926 *U. nuda*, *U. tritici*, *U. hordei*, *U. avenae*, and *U. levis* were collected from widely separated localities, as indicated in table 1. Pure cultures of the smuts were often obtained by transferring

TABLE 1.—*Source of physiologic forms of various cereal smuts*

Species	Form	Source of collection	Year	Collector
<i>Ustilago tritici</i>	1	Minnesota	1925	Writer
	2	Egypt		T. Fahmy
	3	New South Wales		R. J. Noble
	4	Italy		E. Pantanelli
	5	Sweden		T. Lindfors
	6	Bulgaria		D. Atanasoff
	7	Belgium		E. Marchal
	8	Hungary		B. Husz
	9	France	1926	J. Dufrenoy
	10	Michigan	1927	J. J. Christensen
	11	Ohio		do
	12	Kansas		H. E. Parson
	13	Pennsylvania		J. J. Christensen
	14	Wisconsin		do
<i>Ustilago nuda</i>	1	Minnesota (St. Paul)	1925	Writer
	2	Manitoba		do
	3	California		F. W. Briggs
	4	Bulgaria		D. Atanasoff
	5	Belgium		E. Marchal
	6	Hungary		B. Husz
	7	Germany	1926	T. E. Roemer
	8	France		J. Dufrenoy
	9	Sweden	1925	T. Lindfors
	10	Italy		E. Pantanelli
	11	Wisconsin	1927	Writer
	12	Minnesota (Farmington)		do
<i>Ustilago hordei</i>	1	Italy	1925	E. Pantanelli
	2	Minnesota (St. Paul)	1927	Writer
	3	Minnesota (St. Paul)		do
	4	Minnesota (Waseca)		do
	5	Minnesota (Chaska)		E. C. Stakman
	6	Kansas		H. E. Parson
	7	Iowa		R. O. Bulger
<i>Ustilago levis</i>	1	Minnesota (St. Paul)	1927	Writer
	2	Minnesota (Crookston)		do
	3	Virginia		R. M. Nelson
	4	Oklahoma		H. E. Parson
	5	China	1925	C. C. Chen

TABLE 1.—*Cont.*

Species	Form	Source of collection	Year	Collector
<i>Ustilago avenae</i>	1	Minnesota (St. Paul)	1926	Writer
	2	Germany		T. E. Roemer
	3	Italy		E. Pantanelli
	4	France		J. Dufrenoy
	5	Oklahoma (Chickasha)	1927	W. Butler
	6	Oklahoma (Ryan)		do
	7	Oklahoma (Perry)		H. E. Parson
	8	Texas (Greenville)		do
	9	Texas (Dallas)		W. Butler
	10	Kansas (Wichita)		H. E. Parson
	11	Kansas (Manhattan)		E. B. Lambert
	12	South Dakota		R. O. Bulger
	13	Sector from Form 12		
	14	Missouri (Elden)		H. E. Parson
	15	Missouri (Sedalia)		do
	16	Wisconsin (Areadia)		J. J. Christensen
	17	Illinois (Urbana)		do
	18	New York (Ithaca)		do
<i>Tilletia levis</i>	1	Hungary	1925	B. Husz
	2	Minnesota		Writer
	3	Egypt		T. Fahmy
<i>Tilletia tritici</i>	1	New Zealand	1925	G. H. Cunningham
	2	Norway		I. Jorstad

the chlamydospores directly to the culture medium. In other cases chlamydospores were suspended in a 1 per cent solution of copper sulphate for from 12 to 27 hours. Pure cultures then were obtained by transferring the chlamydospores directly to the medium by means of a platinum loop. (See figure 1.) In preliminary tests with several different kinds of media, a 2 per cent potato dextrose agar was found to be the best medium for purposes of studying cultural differences of the physiologic forms of these smuts.

Before final transfers were made for the comparative tests, the pure cultures were grown for a period of two weeks on equal quantities of potato dextrose agar. At the end of this time, with the exception of tests with *U. hordei*, triplicate series of 200 cc. Erlenmeyer flasks containing 35 cc. of 2 per cent potato dextrose agar were inoculated with small, approximately equal portions of each culture. In the comparative tests with *U. hordei*, 150 cc. Erlenmeyer flasks containing 30 cc. of the 2 per cent potato dextrose agar were used. All of the cultures were subjected to the same environ-

mental conditions. The agar was made up in one batch, poured and autoclaved at the same time, and the cultures were grown at room temperature in the laboratory.

The following characters were used in differentiating the forms on culture media: color, topography, character of surface, consistency, and type of margin. The colors were classified according to Ridgway's "Color Standards and Color Nomenclature" (37).

Ustilago tritici. In a preliminary paper, Rodenhiser (39) reported the existence of three physiologic forms of *U. tritici* differentiated on the basis of cultural characteristics. This work has been extended, and 14 distinct forms of *U. tritici*, obtained from the various sources indicated in table 1, have been distinguished on culture media. The data on cultural characteristics of four of these forms are summarized in table 2 and a photograph of them is shown in figure 2. There were striking differences in the cultural characters of the different forms. Many differences in color were observed. For example, form 1 developed an avellaneous color grading to pearl gray in the margin, which may be contrasted with the cartridge-buff to pale olive-gray color of form 10. The cultures of these two forms also differed greatly in topography. Form 1 was umbonate, and it developed fleshy, branched strands which grew in a distinctly counter-clockwise direction. On the other hand, the topography of form 10 was slightly convex, smooth, and scattered over the surface were a few fleshy spines. The surface of the colonies of the different forms ranged from dull to waxy, and the margins from entire to undulating. Under the same environmental conditions the cultural characteristics of any particular form of all the smuts studied were remarkably constant (see figures 7 and 9). Cultural differences were detected, however, only when proper differential materials were used. Dif-

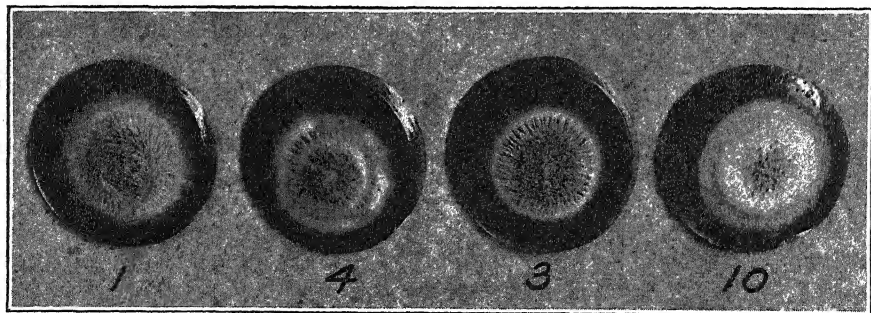


FIG. 2. Four physiologic forms of *Ustilago tritici* grown on potato dextrose agar. Form 1, from Minnesota; form 4, from Italy; form 3, from New South Wales, Australia; form 10, from Michigan.

TABLE 2.—*Cultural characteristics of physiologic forms of Ustilago tritici on potato dextrose agar, 60 days after inoculation*

Source of collection	Form	Color	Topography	Surface	Consistency	Margin
St. Paul, Minnesota Writer	1	Avellaneous to pearl gray in the margin	Umbonate; center covered with fleshy branched strands becoming distinctly counter-clockwise; ray portion smooth	Waxy	Mycelioid and leathery	Entire
New South Wales R. J. Noble	3	Avellaneous to wood brown; margin pearl gray	Convex; complete center coral-like; ray radially furrowed	do	do	do
Italy E. Pantanelli	4	Umbonate portion wood brown grading to light brownish drab; ray pale olive gray	Umbonate with many fine spiney projections; smooth to very slightly furrowed in the ray	Dull	do	Undulating
Williamston, Michigan J. J. Christensen	10	Center cartridge buff; ray pale olive gray	Slightly convex; center smooth with scattered spines	do	do	Entire

ferent forms sometimes looked almost alike on one medium but were entirely different in appearance on another. There also was a great deal of variation in the cultural characters of the same form when grown under different environmental conditions. Similar facts have been observed by Christensen (6 and 7) for *Helminthosporium sativum*, and by Christensen and Stakman (9) for *Ustilago zeae* (Beck.) Ung.

Ustilago nuda. It is evident that there also are distinct physiologic forms of *U. nuda*. Cultures of 12 collections from 12 sources were studied, and all were found to have just as distinct differences in cultural characteristics as those described for *U. tritici*. Cultural characteristics of six of these forms were reported by the writer (39) in a preliminary paper. A detailed description of four of the forms is given in table 3 and a photograph of three forms is shown in figure 3.

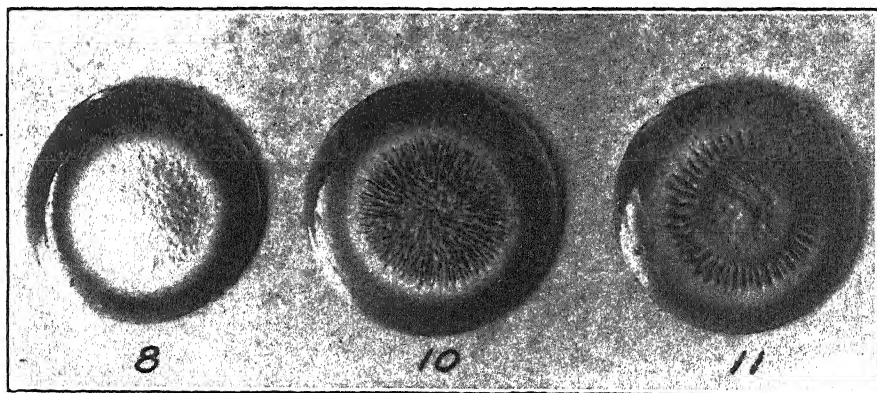


FIG. 3. Three physiologic forms of *U. nuda* grown on potato dextrose agar. Form 8, from France; form 10, from Italy; form 11, from Wisconsin.

The differences in cultural characteristics of different forms of *U. nuda* are sometimes far greater than those between the so-called species *U. tritici* and *U. nuda*. In fact, when grown at 30° C., there is a distinct similarity between the culture of form 6 of *U. nuda* obtained from material collected in Hungary and that of form 2 of *U. tritici* from Egypt (Fig. 4).

All of the morphologic structures of *U. tritici* formed in culture and described by Sartoris (42) were noted. Chlamydospores of both smuts developed in culture. Riehm (38) states that the mycelium of *U. nuda* formed in culture produces straight lateral branches at right angles to the main axis, while in *U. tritici* the lateral branches are curved and more variable in their insertion. The writer, however, observed no consistent differences, such as those described by Riehm. Within the physiologic forms

TABLE 3.—*Cultural characteristics of physiologic forms of Ustilago nuda on potato dextrose agar, 60 days after inoculation*

Source of collection	Form	Color	Topography	Surface	Consistency	Margin
St. Paul, Minnesota Writer	1	Light pinkish cinnamon, with sorghum-brown and smoke-gray patches	Umbonate; slightly felty, with tendency to be smooth at margin; few radial ridges in margin	Chalky	Mycelioid and leathery	Lobate and mycelioid
France J. Dufrenoy	8	Light buff; margin ivory yellow	Umbonate; distinctly felty, margin slightly striated counter-clockwise	Dull to china-like	do	Entire and mycelioid
Italy E. Pantauelli	10	Vinaceous buff; margin pallid neutral gray	Raised; high radial ridges gradually disappearing near the margin	Waxy	do	do
Wisconsin Writer	11	Wood brown, becoming tiller buff at the margin	Convex; center slightly felty with distinct counter-clockwise striations, uniformly deeply furrowed	Waxy to china-like	do	do

of both species all gradations of these morphologic characters were observed. In fact, by changing the environment in which forms of either species are growing, the entire morphological character of the growth may be changed. These variations were brought about by changing the consistency of the culture medium and the kind of medium, and by growing the cultures at different temperatures.

Counter-clockwise growth of mycelium is characteristic of some of the forms at certain temperatures. A study was made of the effect of temperature on this type of growth in four physiologic forms of *U. nuda*, and the

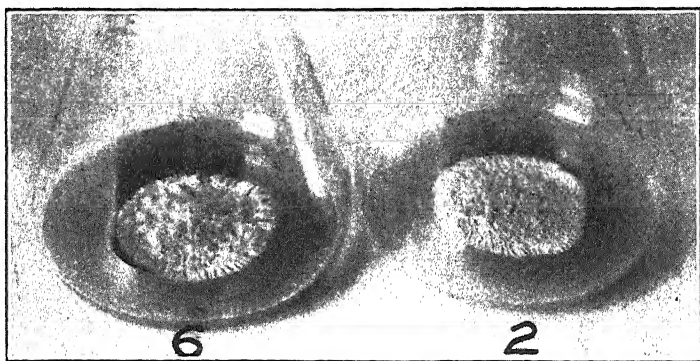


FIG. 4. Twenty-one-day-old cultures of *U. nuda* form 6 and *U. tritici* form 2, grown on potato dextrose agar at 30° C. Note the similarity.

data are summarized in table 4. Counter-clockwise growth of mycelium was very conspicuous when the cultures of form 1 were grown at 15° C., but slightly less so at 20° C. At 10°, 25°, and 30° C., however, the mycelium did not grow in a counter-clockwise manner. No counter-clockwise growth developed in cultures of form 6 at 10° or 15° C., but it did develop at 20° and 25° C.; at 30° C. it developed to a marked degree.

Differences in counter-clockwise growth at different temperatures were also noted in forms 4 and 7. In another series of cultures, form 9, when grown at 20° C., developed a very marked counter-clockwise growth of mycelium. In form 10, on the other hand, growing under identical conditions, the growth was clockwise. (See figure 5.)

Ustilago hordei. Consistent cultural differences were found in seven collections of *U. hordei*. Two forms were collected in the same field at University Farm, St. Paul, Minn., and two more were collected within a radius of one hundred miles from St. Paul. Others were obtained from widely separated localities. There can be no question regarding the distinctiveness of the different physiologic forms in culture. (See figure 6.) Detailed

TABLE 4.—*The influence of temperature on the counterclockwise growth of four physiologic forms of Ustilago nuda*

Form	Temperature in degrees C.				
	10	15	20	25	30
1	0 ^a	+++	++	0	0
4	0	0	+	+	0
6	0	0	+	+	++
7	0	+	+	0	0

^a 0 = absent

+ = present

++ = marked

+++ = very marked

descriptions of four of the seven forms identified are given in table 5. The range of variability of forms of *U. hordei* on culture media is very wide. The character and rate of growth and the ability to produce sporidia are influenced profoundly by environmental conditions. However, when grown under the same environmental conditions, the cultural characteristics are remarkably constant, as shown in figure 7.

Wedge-shaped sectors, which differed from the parents in color and rate of growth, and bred true in subsequent transfers, appeared in some of the cultures. Several explanations may be offered for their occurrence. Since

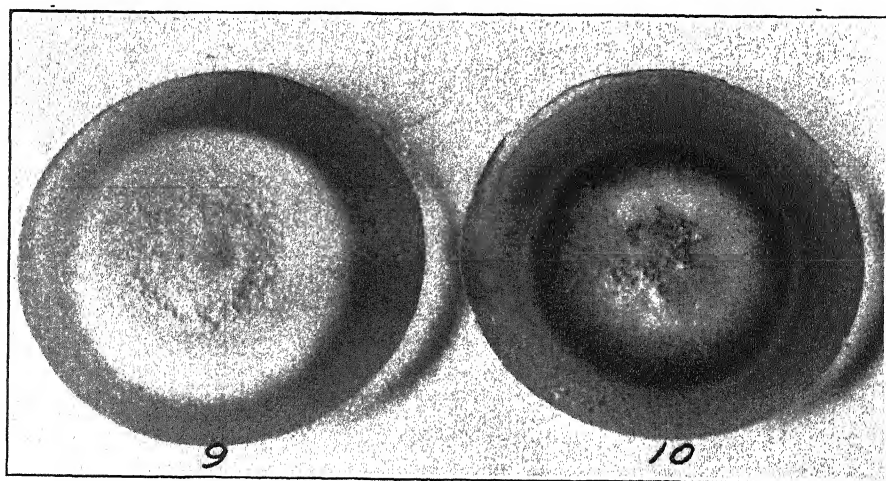


FIG. 5. Two physiologic forms of *Ustilago nuda* grown on potato dextrose agar at 20° C.: form 9, with counter-clockwise growth of mycelium; and form 10, with clockwise growth of mycelium.

the original isolations were not made from single chlamydospores but from a mass culture of a single collection, sectors possibly may have resulted from chance assortment of physiologic forms.

Kniep (28 and 29), Zillig (51), Bauch (2 and 3), Stakman and Christensen (45), and others have shown that there are sexual strains in the smut fungi. Zillig showed that there are sexual strains within the indi-

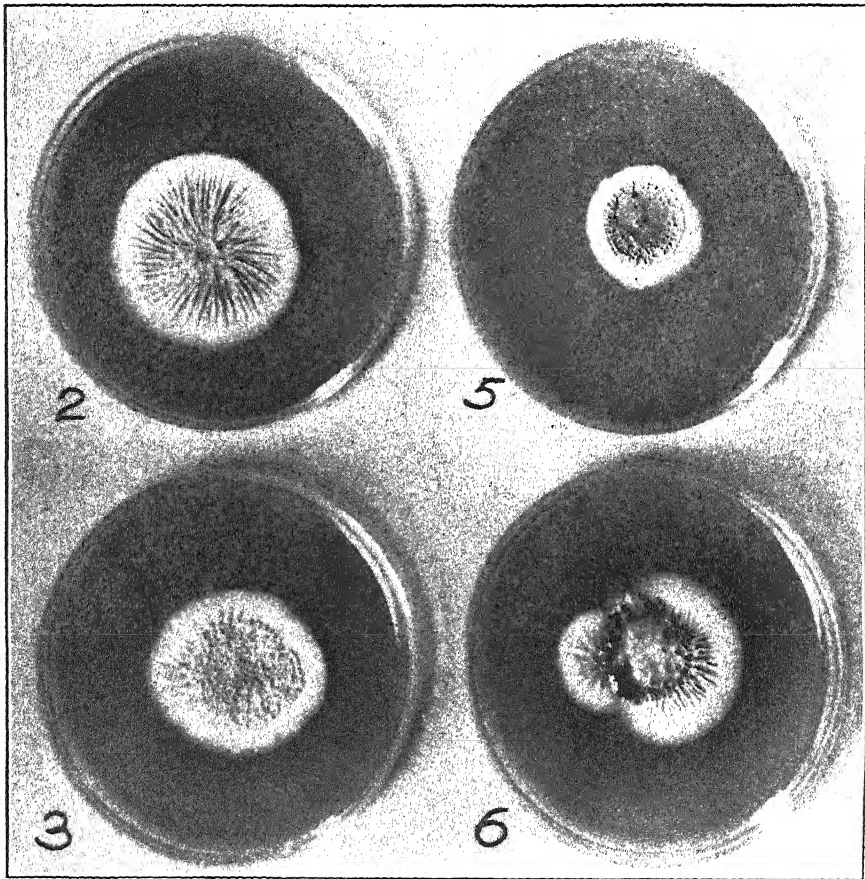


FIG. 6. Four physiologic forms of *Ustilago hordei* grown on potato dextrose agar: forms 2 and 3, from St. Paul, Minnesota; form 5, from Chaska, Minnesota; form 6, from Kansas.

vidual physiologic forms and also that fusions may occur between sexual strains of different physiologic forms. Stakman and Christensen (45) found that sexual strains of *U. zeae* differed in their appearance on culture media. Thus, the sectors might possibly have occurred as a result of segregation for sex. Christensen and Stakman (9) have shown mutation to be

TABLE 5.—*Cultural characteristics of physiologic forms of Ustilago hordei on potato dextrose agar, 24 days after inoculation*

Source of collection	Form	Color	Topography	Surface	Consistency	Margin
Italy E. Pantenelli	1	Vinaceous buff	Convex to pulvinate; center irregularly pitted and rough, felt-like; ray radially ridged	Waxy	Mycelioid and leathery	Undulate and mycelioid
St. Paul, Minnesota Writer	2	Avellaneous to wood brown	Convex; center irregularly pitted, tendency to become reticulate near the ray; ray with conspicuous counter-clockwise ridges	Waxy to dull	do	do
Chaska, Minnesota E. C. Stakman	5	Center wood-brown, changing to light buff in the ray portion	Center raised and coarsely reticulated; ray flat	do	Yeast-like and granular	Lacerate and mycelioid
Carney, Kansas H. E. Parson	6	Light pinkish cinnamon; sprinkling of white	Raised to convex; smooth	China-like	Bacterioid and slimy	do

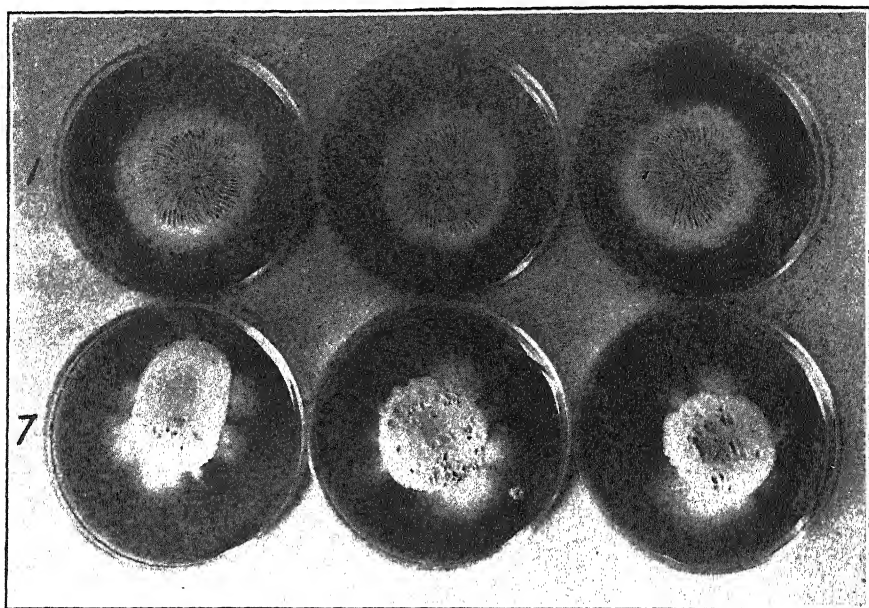


FIG. 7. Cultures showing uniformity of two physiologic forms of *Ustilago hordei* grown on potato dextrose agar: form 1, from Italy; form 7, from Iowa.

common in *U. zeae*. It is possible that these sectors in cultures of *U. hordei* also are the result of mutation. No sectoring such as occurred in *U. hordei* has ever been seen by the writer in cultures of *U. tritici* or *U. nuda*, although these smuts were grown for several years on several different kinds of media and under different environmental conditions.

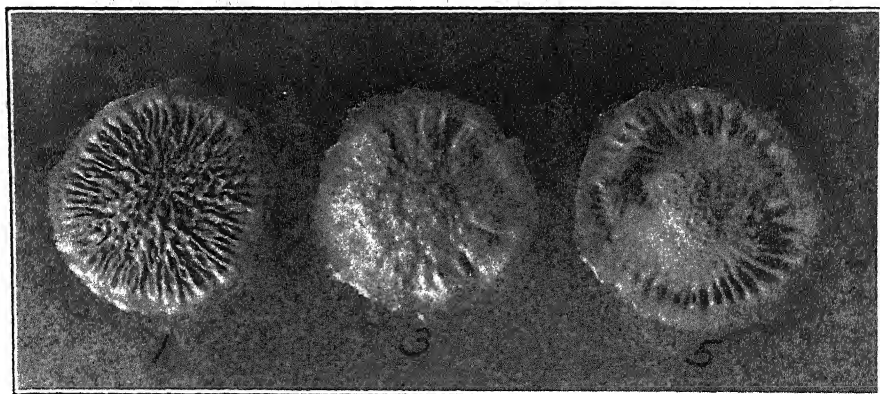


FIG. 8. Three physiologic forms of *U. levis* grown on potato dextrose agar: form 1, from Minnesota; form 3, from Virginia; form 5, from China.

Ustilago levis. Five distinct physiologic forms of *U. levis* were differentiated on the basis of cultural characteristics. Detailed descriptions of three of the forms are given in table 6. There were decided differences in cultural characteristics of these forms (Fig. 8)—in fact greater in some cases than were the differences between cultures of *U. levis* and *U. avenae*. It should be emphasized again that the cultural differences between the forms can be detected only when the proper differential materials are used. Furthermore, there are great differences between forms grown under one particular set of environmental conditions, which are not apparent when the forms are grown in another environment. No consistent microscopic differences were noted in the vegetative growth of *U. levis* and *U. avenae*. No sectors appeared in the cultures of any of the five forms of *U. levis*.

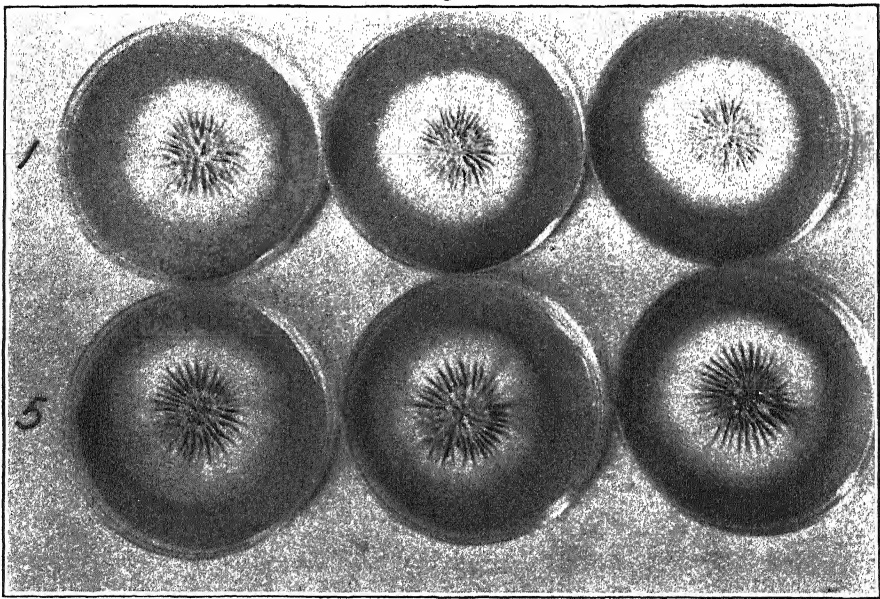


FIG. 9. Cultures showing uniformity of two physiologic forms of *Ustilago levis* grown on potato dextrose agar: form 1, from Minnesota; form 5, from China.

Ustilago avenae. From 22 collections of *U. avenae* that were obtained, 17 physiologic forms were differentiated on the basis of cultural characteristics. Another form was obtained as a result of sectoring in the form originally collected in South Dakota. (See table 1 for the origin of the various forms.) The data on cultural characters of six forms are summarized in table 7. Here again there were decided differences in the cultural characteristics. (See figure 10.)

TABLE 6.—*Cultural characteristics of physiologic forms of Ustilago levis on potato dextrose agar, 24 days after inoculation*

Source of collection	Form	Color	Topography	Surface	Consistency	Margin
St. Paul, Minnesota Writer	1	Smoke gray	Convex; prominent radial ridges of unequal length	Waxy	Mycelioid and leathery	Entire and mycelioid
Virginia R. M. Nelson	3	Pale olive buff to pearl gray	Convex; smooth to warty	Wet, shiny	Bacterioid and slightly mycelioid	Entire, bac- terioid to mycelioid
China C. C. Chen	5	Cream interspersed with mummy-brown	Convex to umbonate; smooth to coarsely ridged; center slightly tufted; slight counter-clockwise growth	Waxy	Mycelioid and leathery	Entire and mycelioid

TABLE 7.—*Cultural characteristics of physiologic forms of Ustilago avenae on potato dextrose agar, 24 days after inoculation*

Source of collection	Form	Color	Topography	Surface	Consistency	Margin
St. Paul, Minnesota Writer	1	Pale olive buff	Raised; mycelioid center 20 mm. in diameter, radially ridged, becoming smooth in ray portion	Wet, shiny	Center mycelioid; ray bacterioid	Lobate; beaded to sparsely mycelioid
Germany T. E. Roemer	2	Buff olive to drab; margin pale gray	Convex to umbonate; numerous daedaloid center - clockwise ridges; margin of short shallow radial ridges	Waxy	Mycelioid and leathery	Appressed, entire and mycelioid
Arcadia, Wisconsin J. J. Christensen	16	Prout's brown; margin of 5 mm. pearl gray	Convex to umbonate; numerous chain-like radial ridges, irregular in size and length	Moist to waxy	do	do
Chickasha, Oklahoma W. Butler	5	Mummy brown center 20 mm. diameter, ivory in ray portion; margin pearl gray to yellow	Slightly umbilicate with high compact radial ridges	Center glossy; ray waxy	Center chiefly bacterioid; remainder mycelioid and leathery	Mycelioid and plumose
Dallas, Texas W. Butler	9	Light buff to pale gull gray	Umbonate; center of upright tufts; numerous shallow irregular fine chain-like ridges	Wet, shiny	Mycelioid and leathery	Slightly waxy and mycelioid
Sector from form 12	13	Center of 8 mm. light pinkish cinnamon; ray portion mummy-brown; margin pearl gray	Convex; center crater-like, sparsely tufted; ray portion of regular narrow ridges	do	do	Appressed, mycelioid, plumose

With one exception, the type of growth and ability to produce sporidia could be influenced easily by changing the environmental conditions. In the original transfer, form 2 produced sporidia abundantly. However, in as many as 25 subsequent transfers to several different kinds of media, containing different amounts of moisture and stored at different temperatures, cultures of this particular form consisted almost entirely of mycelium. Forms 11 and 12 produce sporidia more abundantly than do any of the other forms, but they can always be made to produce some mycelium by decreasing the amount of moisture in the culture media.

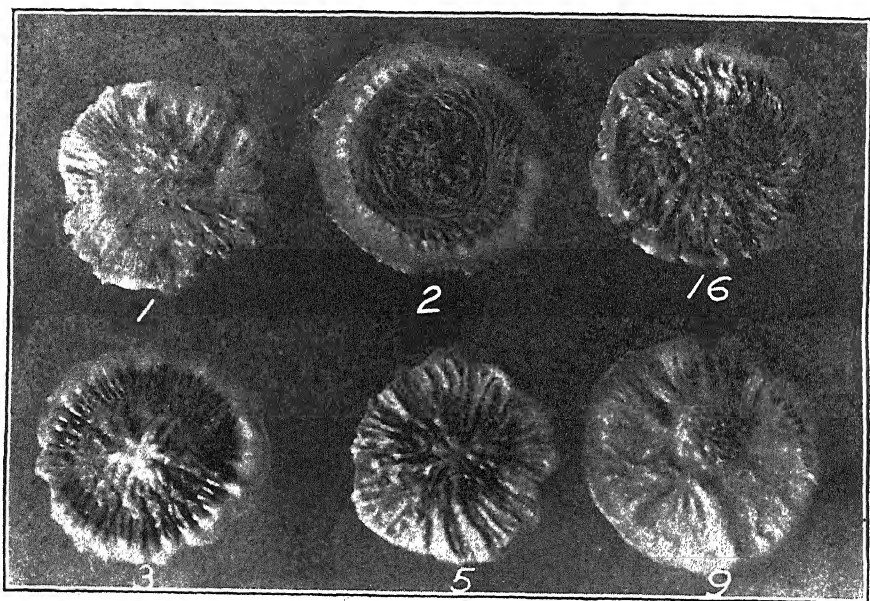


FIG. 10. Six physiologic forms of *Ustilago avenae* grown on potato dextrose agar: form 1, from Minnesota; form 2, from Germany; form 16, from Wisconsin; form 13, from sector of form 12; form 5, from Oklahoma; form 9, from Texas.

EFFECT OF TEMPERATURE ON THE GROWTH OF PHYSIOLOGIC FORMS

Differences in the way in which cultures of physiologic forms of various fungi react to temperature have been noted previously. Edgerton (13) was able to distinguish between two physiologic forms of *Glomerella cingulata* (Atk.) S. and S., by means of temperature relations.

Similar differences were shown between strains of *Rhizopus nigricans* Ehrnb., by Harter and Weimer (21). One strain of *R. nigricans* was found to have a lower optimum than seventeen others. Johnson (27) found different temperature requirements for two physiologic forms of *Helmintho-*

sporum gramineum Rab. No appreciable differences of growth occurred at the optimum temperature, which was the same for both forms, but one grew decidedly better than the other at low temperature (5-6° C.). No such differences have been shown between cultures of physiologic forms of the smut fungi.

In order to determine whether the physiologic forms of some of the smuts could be distinguished on the basis of reaction to temperature, a series of tests was made as follows: To each of a triplicate series of 200 cc. Erlenmeyer flasks were added 35 cc. of the same preparation of 2 per cent potato dextrose agar. The flasks were then inoculated with small, but approximately equal, portions of each culture and all incubated at room temperature for a period of 24 hours. Thus the different forms were subjected as nearly as possible to identical environmental conditions before distribution to various temperatures. All of the forms were incubated simultaneously at each of the following temperatures: 10°, 15°, 20°, 30°, and 35° C. The diameter of each colony was measured at the end of 14 days and again at the end of 21 days. Differences in rate of growth were apparent at the end of 14 days but were more striking at the end of 21 days. The figures given are therefore based on the diameters of the colonies at the end of 21 days. As there was very little difference in the size of colonies in replicated cultures of some forms, only the averages are recorded in the tables. In order to ascertain whether the physiologic forms would react the same to different temperatures after a period of time, the experiments made with *U. tritici* and *U. nuda* in 1927 were repeated one year later. During this time transfers had been made to fresh media six different times and the cultures subsequently were stored under the same environmental conditions.

Ustilago tritici. The results of the experiment with four physiologic forms of *U. tritici* are summarized in table 8 and the data obtained in 1927 are graphically represented in figure 11. It seems clear from table 8 and figure 11 that there are differences in the reactions of some physiologic forms of *U. tritici* to temperature. There were differences in the optimum temperature for growth, as indicated by the size of colonies and also in the total amount of radial growth at certain temperatures. (See figure 12.) The optimum temperature for form 4 was 20° C. For forms 1, 2, and 5, it was 25° C. Form 2 is distinct from forms 1, 4, and 5 in that it has a wider temperature range and also the amount of radial growth attained by it is greater at all temperatures. In 1927, at the end of 21 days incubation at 35° C., the average diameter of the colonies of form 2 was 6.7 mm., while cultures of forms 1, 4, and 5 failed to grow at all. There was a difference of 7 mm. in the diameter of growth between forms 1 and 2 at 10° C. The differences in the amount of radial growth are not so marked between forms

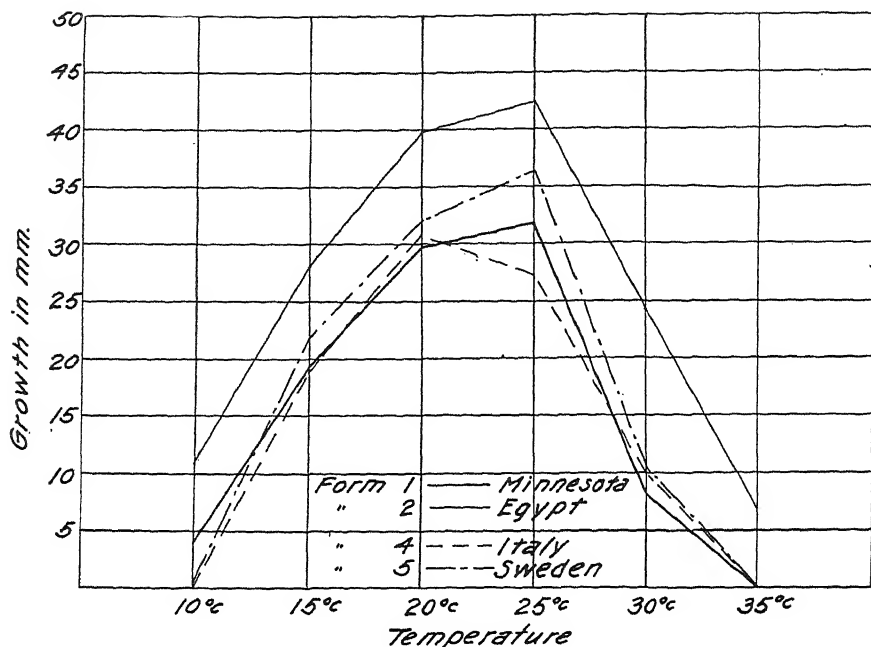


FIG. 11.—The effect of temperature on the growth of four physiologic forms of *Ustilago tritici* grown on potato dextrose agar for 21 days, in 1927.

1 and 5 as between some of the other forms. At 20° C. there is a difference of only 3.3 mm. in the diameter of the colonies, while at 25° C., the optimum

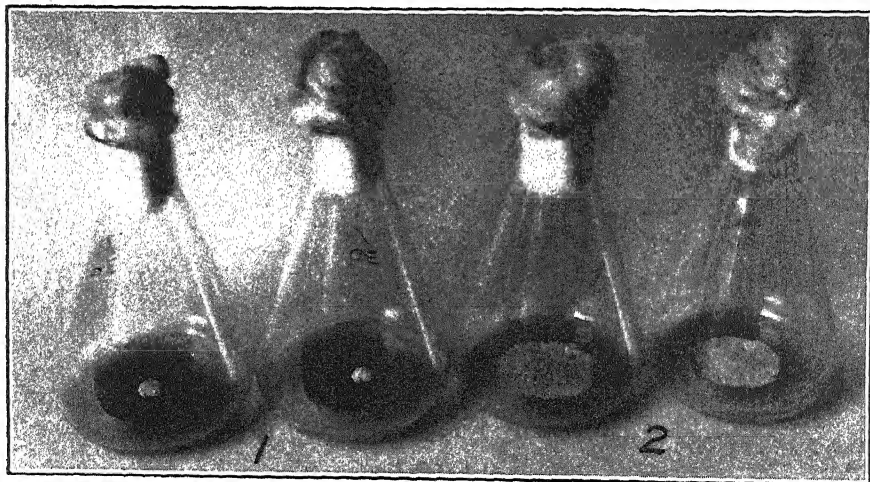


FIG. 12.—Two physiologic forms of *Ustilago tritici* grown on potato dextrose agar at 30° C.: form 1, from Minnesota; form 2, from Egypt.

temperature for their growth, this difference is slightly increased to 4.6 mm. Form 4 is distinct from forms 1 and 5 in that it has a different optimum temperature for growth.

The experiments which were made with the various physiologic forms of *U. tritici* in October, 1927, were repeated in October, 1928. During this period the cultures were transferred several times at 60-day intervals to fresh media, once to 2 per cent malt-extract agar and five times to 2 per cent potato dextrose agar. When again tested in 1928 for differences in their reaction to temperature, they behaved in the same manner as they had in 1927. The diameter of the colonies in 1928 was slightly less in all cases than that in 1927, but this may have been due to a very slight difference in the media used in the two years.

Ustilago nuda. Five physiologic forms of *U. nuda* were tested for differences in their growth reactions at the various temperatures noted above. The data obtained are summarized in table 9, and the 1927 results are represented graphically in figure 13. It is evident that there are differences in the reaction to temperature of physiologic forms of *U. nuda*. (See figures 14 and 15.) There were differences in the optimum temperature for growth of certain physiologic forms and differences in the amount of radial growth at certain temperatures. The optimum temperature for

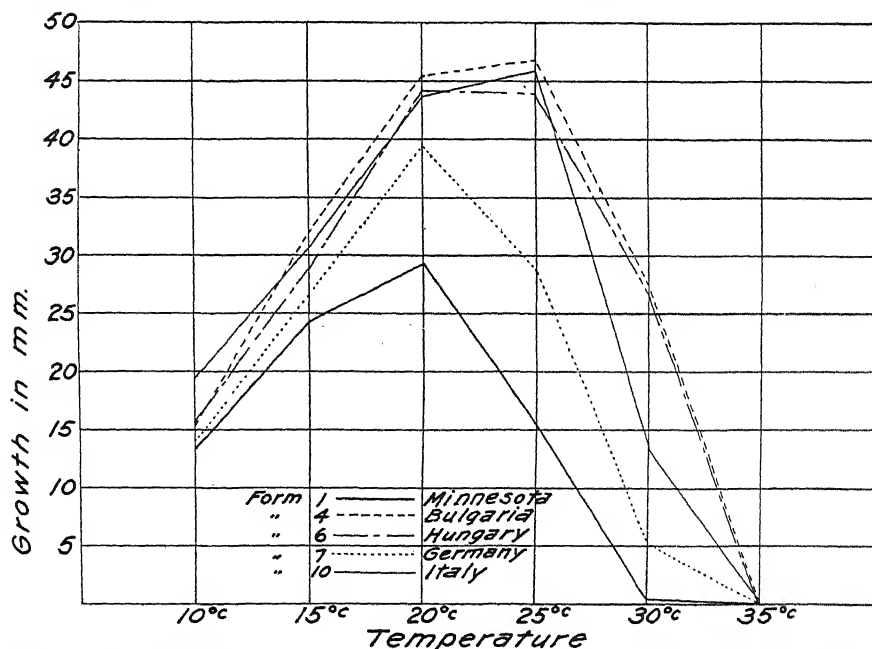


FIG. 13. The effect of temperature on the growth of five physiologic forms of *Ustilago nuda* grown on potato dextrose agar for 21 days, in 1927.

TABLE 9.—*The influence of temperature on the growth of five physiologic forms of Ustilago nuda grown on potato dextrose agar for 21 days*

Temperature in degrees C. and diameter of colonies in mm.																			
10			15			20			25			30			35				
Form number	Series ^a			Series			Series			Series			Series			Series			
	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	
1	13.3	8.7	11.0	24.3	17.3	20.8	29.2	22.3	25.8	15.3	16.0	15.7	Tr.	0.0	Tr.	0.0	0.0	0.0	
4	15.5	12.3	13.9	32.0	24.3	28.2	45.5	40.7	43.1	46.7	45.7	46.2	27.0	23.0	25.0	Tr.	0.0	Tr.	
6	15.7	12.7	14.2	28.8	24.0	26.4	44.2	35.0	39.6	44.0	32.3	38.2	26.7	26.3	26.5	0.0	0.0	0.0	
7	13.7	13.7	26.8	26.8	39.5	39.5	28.7	28.7	5.2	5.2	0.0	0.0	
10	16.3	15.0	15.7	30.7	31.0	30.9	43.7	41.7	42.7	45.8	46.7	46.3	13.2	20.3	16.8	Tr.	0.0	Tr.	

^a Series 1 run in October, 1927; Series 2 in October, 1928.

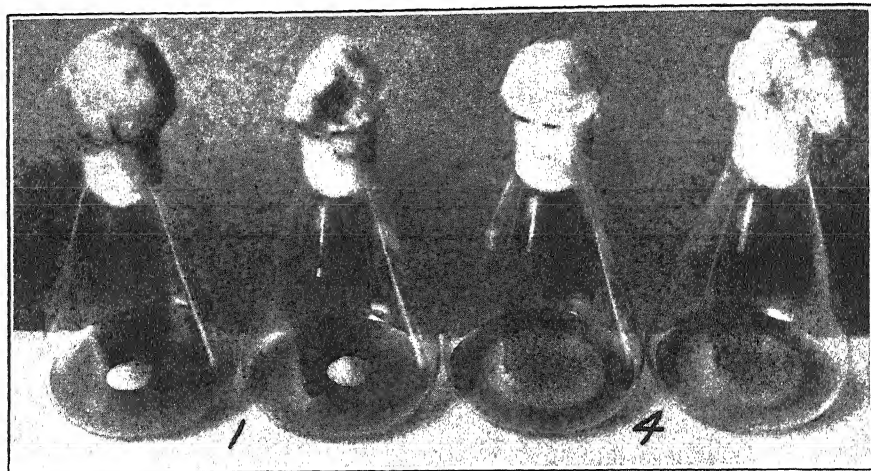


FIG. 14. Two physiologic forms of *Ustilago nuda* grown on potato dextrose agar at 25° C.: form 1, from Minnesota; form 4, from Bulgaria.

growth of forms 1 and 7 was 20° C. and that of forms 4 and 10, 25° C. Although forms 1 and 7 have the same optimum temperature for growth, they are distinctly different as regards the amount of radial growth at certain temperatures. In the 1927 tests these two forms grew about equally well at 10° C. At 20° C., however, the culture of form 7 grew, on the average, 10.3 mm. in diameter more than did form 1. Likewise, at 25° C. the difference in diameter of the colonies of these two forms was 13.4 mm.

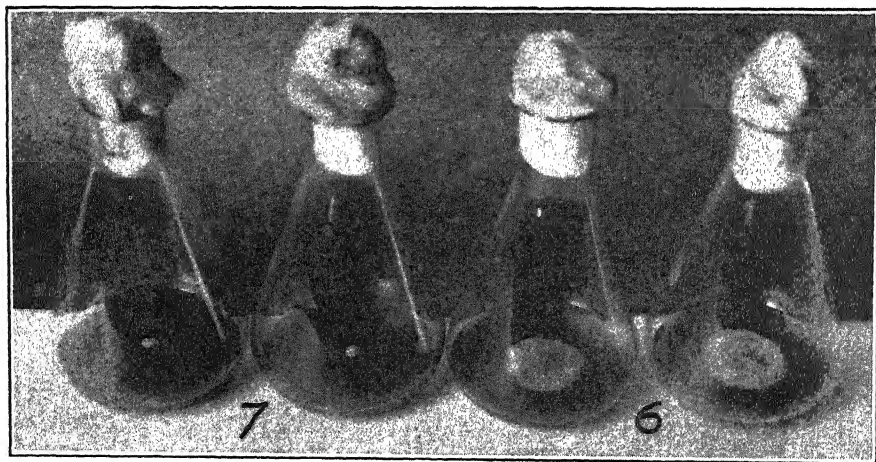


FIG. 15. Two physiologic forms of *Ustilago nuda* grown on potato dextrose agar at 30° C.: form 7, from Germany; form 6, from Hungary.

At 30° C. the differences were less marked, and at 35° C. neither form grew at all. The differences in the amount of radial growth made by forms 4, 6, and 10 were very slight and evidently not significant. As a group, however, they reacted distinctly differently from forms 7 and 1. At 10° and 15° C. the differences in the amount of growth of all the forms was not great, but at 25° C. the differences between forms 4, 6, and 10 as a group, and forms 7 and 1, were very wide. The average diameter of the colonies of form 1 at this temperature was 15.3 mm.; form 7, 28.7 mm.; and of forms 4, 6, and 10, it was 46.7, 44.0, and 45.8 mm. respectively.

The cultures of the physiologic forms of *U. nuda* tested in October, 1927, for differences in their reaction to temperature were again tested in October, 1928. They were transferred to fresh media and stored as described for similar tests with physiologic forms of *U. tritici*. Here again the results obtained in 1928 agreed with those obtained the previous year. The cultural characteristics of any one physiologic form of the smuts tested were found to be very constant when the cultures were grown at the same temperature. By changing the temperature, however, variations were induced, particularly in the topography of the colonies.

Ustilago hordei. It seems clear from table 10 and figure 16 that physiologic forms of *U. hordei* react differently when grown at certain tempera-

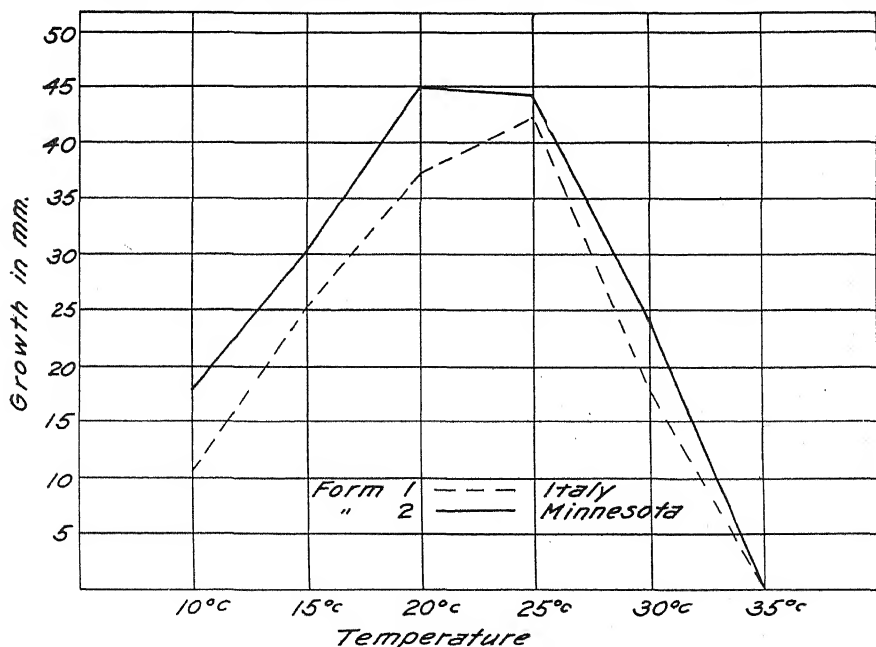


FIG. 16. The effect of temperature on the growth of two physiologic forms of *Ustilago hordei* grown on potato dextrose agar for 21 days, in 1928.

TABLE 10.—*The influence of temperature on the growth of two physiologic forms of Ustilago hordei grown on potato dextrose agar for 21 days*

Temperature in degrees C. and diameter of colonies in mm.																		
Form number	10			15			20			25			30			35		
	Series			Series			Series			Series			Series			Series		
	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.
1	9.0	10.0	10.5	23.0	27.7	25.4	35.7	38.7	37.2	42.0	42.5	42.3	17.7	18.0	17.9	0.0	0.0	0.0
2	15.7	18.0	16.9	30.0	31.3	30.2	44.3	45.5	44.9	42.7	46.0	44.4	23.0	25.3	24.2	0.0	0.0	0.0

tures, as did the forms of *U. tritici* and *U. nuda*. Only two forms were used in the test, but it is very probable that, had this number been increased, more differences would have been found.

Form 1 has an optimum temperature somewhere between 20° and 25° C., while that of form 2 is 25° C. At all temperatures between 10° and 30° C. the diameter of the colonies of form 2 was greater than that of form 1. At 25° C., however, the difference in the diameter was only 2.1 mm. while at 20° C. this difference was widened to 7.7 mm. At 35° C. cultures of neither form developed. Series 1 of this experiment was run in November, 1927. Two months later the experiment was repeated. In the meantime the cultures of the two physiologic forms had been transferred at intervals of two weeks to a 2 per cent malt extract, then to 2 per cent malt agar, and finally to a 2 per cent potato dextrose. The reaction of the physiologic forms to temperature in the second series was similar to that in the first series.

Ustilago levis and *Ustilago avenae*. Three physiologic forms of *U. levis* and four of *U. avenae* were tested for differences in reaction to temperature. The differences in the amount of radial growth of physiologic forms of both species were very slight and evidently fall within the limits of experimental error.

Physiologic forms of some of the smut fungi can thus be differentiated by differences in the optimum temperature for growth and in the amount of growth attained at certain temperatures. This is not only interesting but may be of practical importance. The fact that there are these differences may account for the development of a particular physiologic form in a certain geographical area. Furthermore, it may account for variation from year to year in the susceptibility to smut of certain varieties of grain.

Fromme (17) reported that the percentages of wheat heads affected with *U. tritici* varied according to the degree of soil fertility maintained. He concluded that it seems probable that these variations are due to the total or partial elimination of the fungus by the greater vigor of the growth of the plants on the more fertile soil. It would be interesting to know, then, what the results would be if the temperature factor were controlled and a vigorously growing physiologic form of *U. tritici* were used, as for example form 2, which was collected in Egypt.

PATHOGENIC DIFFERENCES BETWEEN PHYSIOLOGIC FORMS AND VARIETAL
RESISTANCE OF WHEAT, BARLEY, AND OATS TO SMUT

Varietal resistance of spring wheat to Tilletia levis

In connection with the study of the pathogenicity of physiologic forms of bunt, a study was made of the varietal resistance of spring wheat to

Tilletia levis Kühn. Heretofore much of the work on bunt resistance has been done with winter wheat varieties. Woolman and Humphrey (50) summarized the literature up to 1924 on varietal resistance to bunt. Heald and Woolman (22), Stephens and Woolman (48), Coons (10), Johnston (25 and 26), and Gaines (18 and 19) have also recently reported on varietal resistance to bunt.

In 1919, about 870 varieties and strains of spring wheats were tested for varietal resistance to *T. levis* in Minnesota. This number was reduced from year to year until by 1923 all except about 40 of the most important varieties had been dropped. The results were reported by Stakman, Lambert, and Flor (46). They concluded that the durum, as a class, were more resistant than the common wheats, but that there were wide differences within both classes.

The varieties used by Stakman *et al.* (46), in 1923, were tested for varietal susceptibility by the writer from 1924 to 1927 inclusive. The seed was very heavily smutted artificially a few days before sowing, and each variety was grown in triplicate rod rows systematically distributed throughout the plot. Shortly after harvest, 200 heads were selected at random from the rod row bundles of each variety. The percentage of bunted heads was then determined from this number.

The results presented in table 11 indicate that there is considerable variation in the amount of bunt developed within the individual groups of wheat. There appears, nevertheless, to be a general correlation between the species group and the degree of resistance. Eighteen varieties and selections of *Triticum vulgare* were grown, and the average percentage of bunted heads was 27.1. In eighteen varieties and selections of durum the average was 7.6 per cent; for two varieties of emmer, 12.3 per cent; and for one variety of einkorn there was an average of 23.6 per cent of bunt. Einkorn, however, is much more resistant than this percentage seems to indicate, for in practically every case the heads were only partially smutted.

There was considerable difference in the amount of bunt in the varieties of common wheat. Marquis, C. I. 3641, with 7.9 per cent of bunt, was the most resistant during the period of four years. Parker, Minn. 2222, Marquillo, C. I. 6887, and Quality, C. I. 6607, had 8.7, 9.5, and 9.7 per cent of bunt respectively and are apparently in the same class with Marquis. Ceres, C. I. 6900, appeared most susceptible, with 52.8 per cent of bunt. Kota, C. I. 5878, and Progress, C. I. 6902, were practically the same with 52.6 and 50.7 per cent respectively.

Of the durum wheats, Kubanka, C. I. 2094, with 2.4 per cent of bunt, and Akrona, C. I. 6881, with 3.2 per cent, were the most resistant varieties. Arnautka, C. I. 1431, with 15.5 per cent of bunt, and Acme, C. I. 5284, with

13.2 per cent, were most susceptible. Many of the heads of the durum varieties were only partially smutted.

The fact that there are resistant varieties in each group of wheat, that is, in the 42-, the 28-, and 14-chromosome groups, indicates that resistance is not correlated with any particular morphologic type. It should then be possible to breed varieties of wheat which are desirable agronomically and which are highly resistant to bunt. Gaines (19) already has called attention to these facts and has developed at least one good wheat, Ridit, which for a time was immune from *Tilletia tritici* in the Palouse district of the West. The results of the tests recorded in table 11 indicate that the production of bunt-resistant, hard-red common wheats should not be difficult. The question arises, however, as to whether those varieties which are resistant to bunt in one locality will be resistant to bunt which occurs in other geographical areas. In general the percentages of bunt obtained by the writer were much higher than those obtained by Stakman, Lambert, and Flor (46). In tests made each year from 1919 to 1923 these investigators obtained an average of only 0.7 per cent of bunt in Marquis, C. I. 3641. Using the same variety each year from 1924 to 1927, the writer obtained an average of 7.9 per cent. In similar comparisons the average percentage of bunt in Kota, C. I. 5878, was increased from 16.5 to 52.6; in Pentad, C. I. 3822, from 0.3 to 8.9; and in Arnautka, C. I. 1431, from 2.4 to 15.5. Einkorn, C. I. 2433, when tested by Stakman *et al* was found to be immune to bunt, but in the writer's tests an average of 23.6 per cent developed.

Increases in the percentage of bunt of wheat in 1924 were not confined to varieties grown at University Farm, St. Paul, Minnesota. During the summer of 1924 there was an unusually high percentage of bunt in Marquis and in durum wheats throughout the entire hard red spring wheat region. The following summer there was an unusual outbreak of bunt, caused, in Minnesota, at least, principally by *T. levis*, although *T. tritici* was found to some extent. The outbreak might be accounted for in two ways: either the weather and soil conditions were unusually favorable for the development of bunt or there may have been an unusually virulent strain of the pathogene. The latter explanation seems the more probable because bunt has continued to be epidemic in the durums since the first outbreak. Furthermore, Faris (15) obtained some preliminary evidence of differences in pathogenicity of *T. levis* and *T. tritici*. Work was therefore undertaken to ascertain whether there actually are distinct physiologic forms of *T. levis* and *T. tritici*. A preliminary paper was published by Rodenhiser and Stakman (40) on the results obtained in 1926. The work was extended in 1927 and the data for both years are given in this paper.

TABLE 11.—*The percentage of bunt (Tilletia levis) on wheat varieties at University Farm, St. Paul, Minnesota, in 1924-1927*

Species and varieties	C. I. Number	Year and percentage of smutted heads				
		1924	1925	1926	1927	Average
<i>Triticum vulgare</i>						
Ceres	6900	—	63.5	41.0	54.0	52.8
Glyndon Fife	2873	54.0	44.8	29.2	45.7	43.4
Hard Federation	4733	34.0	16.2	17.2	40.3	26.9
Haynes Bluestem	2874	39.0	23.0	15.8	12.0	22.5
Kitchener	4800	28.0	36.6	20.6	37.2	30.6
Kota	5878	52.0	56.2	35.7	66.3	52.6
Marquillo	6887	15.1	6.5	6.0	10.5	9.5
Marquis	3641	9.0	8.2	3.2	11.0	7.9
Marquis (Parker's) Minn. 2222	—	—	3.5	12.0	10.5	8.7
Power	3697	—	13.0	21.5	3.0	12.5
Prelude	4323	43.0	23.7	39.7	61.2	41.0
Preston	2958	26.0	7.5	12.0	16.2	15.4
Preston	3021	22.0	30.2	20.8	26.3	24.8
Progress	6902	—	49.5	30.5	72.0	50.7
Quality	6607	—	14.5	4.0	10.5	9.7
Red Bobs	2157	47.0	27.6	22.2	40.0	34.2
Ruby	2135	31.0	17.2	10.3	29.3	22.0
Stanley Fife	1594	18.0	28.8	10.5	32.3	22.4
Average						27.1
<i>Triticum compactum</i>						
Washington little club	4066	—	—	—	67.5	67.5
<i>Triticum durum</i>						
Acme	5248	13.0	6.5	8.2	25.0	13.2
Akrona	6881	—	4.0	1.0	4.5	3.2
Arnautka	1431	18.0	15.8	7.0	21.3	15.5
Arnautka	1494	11.0	3.8	1.0	11.2	6.8
Arnautka	1537	5.0	5.5	0.2	10.0	5.2
Bolley's D 7	3323	14.0	3.7	6.2	15.8	9.9
Iumillo	1736	7.0	1.5	0.3	10.5	4.8
Kahla	2088	8.0	1.3	5.0	6.0	5.1
Kubanka	1354	4.0	3.0	1.7	—	2.9
Kubanka	1516	11.0	9.5	3.2	28.3	13.0
Kubanka	1440	—	11.3	5.7	22.2	13.1
Kubanka	2094	3.0	0.7	1.2	4.5	2.4
Kubanka	2234	4.0	2.8	1.0	10.3	4.5
Kubanka	2952	3.0	3.7	0.3	9.0	4.0
Mindum	5296	6.0	1.3	0.5	18.5	6.6
Nodak	6519	—	3.5	7.0	11.0	7.2
Monad	3320	14.0	4.2	2.6	19.0	10.0
Pentad	3822	13.0	3.0	5.7	13.7	8.9
Average						7.6
<i>Triticum dicoccum</i>						
Khapli	4013	7.0	2.1	5.5	18.7	8.3
White Spring Emmer	1524	11.0	13.8	0.5	38.7	16.0
Average						12.3
<i>Triticum monoceum</i>						
Einkorn	2433	19.0	33.8	5.8	35.7	23.6

Pathogenicity of physiologic forms of Tilletia levis and Tilletia tritici

Five collections of *T. levis* and seven of *T. tritici* were obtained from the places indicated in tables 12 and 14.

In the spring of 1925 Kota wheat, C. I. 5878, was inoculated with each collection of smut, except those from California and Washington which had not yet been obtained. The smutted heads which resulted were then picked and kept under the same conditions until the spring of 1926. Likewise, the inoculum used in the spring of 1927 was that which had developed on Kota wheat the previous year and was then stored under the same conditions until used in 1927. Thus the spores of all of the collections, with the exception mentioned, were produced and kept under similar conditions.

Mindum, C. I. 5296, a durum wheat; Einkorn, C. I. 2433; Marquis, C. I. 3641, and Kota, C. I. 5878, both hard red spring wheats, were inoculated with chlamydospores of each collection of smut. In 1927, Pentad, C. I. 3822, a durum wheat, was substituted for the Mindum used in 1926. Previous to inoculation, the seed was treated by Jensen's modified hot water method. When thoroughly dry, the seed was inoculated with powdered inoculum from each collection, at the rate of 0.5 grams to 100 grams of seed. All the seed was then sown on the same day, in 1926, in duplicate and in 1927 in triplicate systematically distributed rod rows. As there was very little difference in the percentage of smut in the replicated rows, only the averages are recorded.

With one exception, the percentages of infection obtained in 1926, as given in tables 12 and 14, are based on counts of 800 heads of Einkorn, 800 of Marquis, and 1000 of Kota. The counts on the varieties inoculated with the Egyptian collection are based on counts of 400 heads of Einkorn, 400 of Marquis, and 500 of Kota. Each individual head of wheat was cut in order to detect partial smutting. The percentages of partially and completely smutted heads of Einkorn and Marquis were recorded separately. In 1926 the number of partially smutted heads of Kota was not determined, because there was very little partial smutting. So little smut developed in Mindum that the results are not recorded. The percentages of infection obtained in 1927, recorded in tables 13 and 15, are based on counts of 900 heads of each variety. Three hundred heads were selected at random from each of the triplicated rod rows, but here again the differences in the percentage of smut in the replicated rows was so small that averages only are recorded.

The results of the two-year tests are recorded in tables 12 to 15 inclusive and the data obtained in 1926 are presented graphically in figures 17 and 18. The varieties of wheat used in the tests have been grown at University Farm, St. Paul, Minn., for eight years, from artificially smutted seed, and

with one exception there has always been a high degree of correlation between the percentage of bunt in the different years, as well as in the replicated rod rows. During the last four years, however, a much higher percentage of bunt has developed in practically all of the varieties than in the previous seasons.

It is evident from figures 17 and 18 and tables 12, 13, 14, and 15, that there are sufficiently great differences in the virulence of several collections of smut to justify the conclusion that there are physiologic forms which differ in pathogenicity. In table 16 is indicated the relative susceptibility of each variety to each of these forms in 1926. It seems clear from these tables that there are at least three physiologic forms of *T. levis*. The two collections from Hungary were so nearly alike in 1926 that they are considered to be identical and are designated as form 1. In comparison with the behavior of form 1, in 1926, the collection from Minnesota was slightly more virulent on Einkorn, about the same on Marquis, but was less virulent on Kota.

In 1927 similar results were obtained, with the exception that the Minnesota collection was somewhat less virulent than form 1 on Marquis. In an additional test with Pentad, in 1927, this collection proved to be less virulent than form 1. It is considered as form 2. The collection from Egypt, when tested in 1926, was more virulent on Einkorn than the other two, decidedly less virulent on Marquis, and somewhat less virulent on

TABLE 12.—*The percentage of smutted heads in Marquis and Kota wheats and in Einkorn inoculated artificially with five collections of Tilletia levis at University Farm, St. Paul, Minn., in 1926*

Source of inoculum	Germination of chlamydo-spores in per cent	Percentage of smutted heads						
		Einkorn			Marquis			Kota
		Partial	Complete	Total	Partial	Complete	Total	
Minnesota	65	8.4	6.7	15.1	0.4	7.1	7.5	64.3
Hungary ^a	80	12.7	1.9	14.6	0.9	4.6	5.5	61.7
Hungary ^b	75	7.4 ^c	1.5	8.9	1.7	5.3	7.0	61.1
Italy	80	13.4	7.3	20.7	1.2 ^c	0.2	1.4	56.7
Egypt	90	9.8	8.3	18.1	0.3 ^c	0.3	0.6	37.6
Uninoculated	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0

^a Hatvan

^b Debreizen

^c Individual heads lightly smutted. Only a few smutted, or partially smutted kernels in each hand.

TABLE 13.—*The percentage of smutted heads in Marquis, Kota, and Pentad wheats and in Einkorn inoculated artificially with four collections of Tilletia levis at University Farm, St. Paul, Minn., in 1927*

Source of inoculum	Germination of chlamydo-spores in per cent	Percentage of smutted heads									
		Einkorn		Marquis		Kota		Pentad			
		Partial	Complete	Partial	Complete	Partial	Complete	Partial	Complete	Partial	Total
Minnesota	85	30.5	1.0	3.4	1.9	5.3	14.5	2.9	0.4	2.9	3.3
Hungary ^a	80	22.4	1.6	7.2	4.0	11.2	18.3	4.6	0.9	4.6	5.5
Italy	60	41.8	5.9	1.9	0.5	2.4	18.4	4.3	1.2	4.3	5.5
Egypt	70	46.6	8.0	0.3	0.1	0.4	24.0	10.9	4.1	10.9	15.0
Uninoculated	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

^a Debreizen.

Kota. In 1927 similar results were obtained except that this collection was more virulent on Kota. When tested on Pentad in 1927, it was found to be decidedly more virulent than either form 1 or 2. This collection therefore is designated as form 3. The collection from Italy is somewhat similar to the one from Egypt, although it is much less virulent on Pentad. This may be another form, but until further tests are made it is not so considered.

One might conclude that there are several different forms of *T. tritici* (tables 14 and 15). The New Zealand collection was consistently less virulent in both the 1926 and 1927 tests than that from Norway; and the differences are so great that one is forced to the conclusion that they represent two distinct forms. The collection from New Zealand is therefore desig-

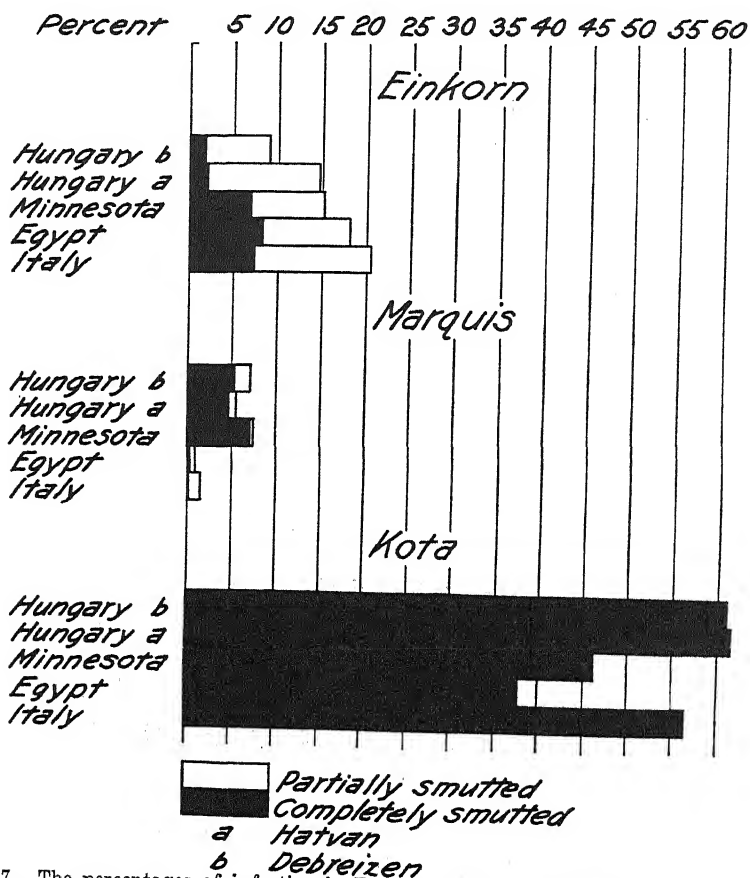


FIG. 17. The percentages of infection in Einkorn, Marquis, and Kota inoculated with five collections of *Tilletia levis*, in 1926.

nated as form 1 and the one from Norway as form 2. For the present the collection from Hungary is considered to be form 2. The collection from Washington is similar to form 1 on Marquis, Kota, and Pentad but is more virulent on Einkorn. It probably is a third form, but until this collection, as well as those from Sweden, Manitoba, and California, are tested on other varieties of wheat, no definite form numbers are assigned to them.

The results of these tests indicate that, although one may breed varieties of wheat which are highly resistant to bunt in one locality, there is no as-

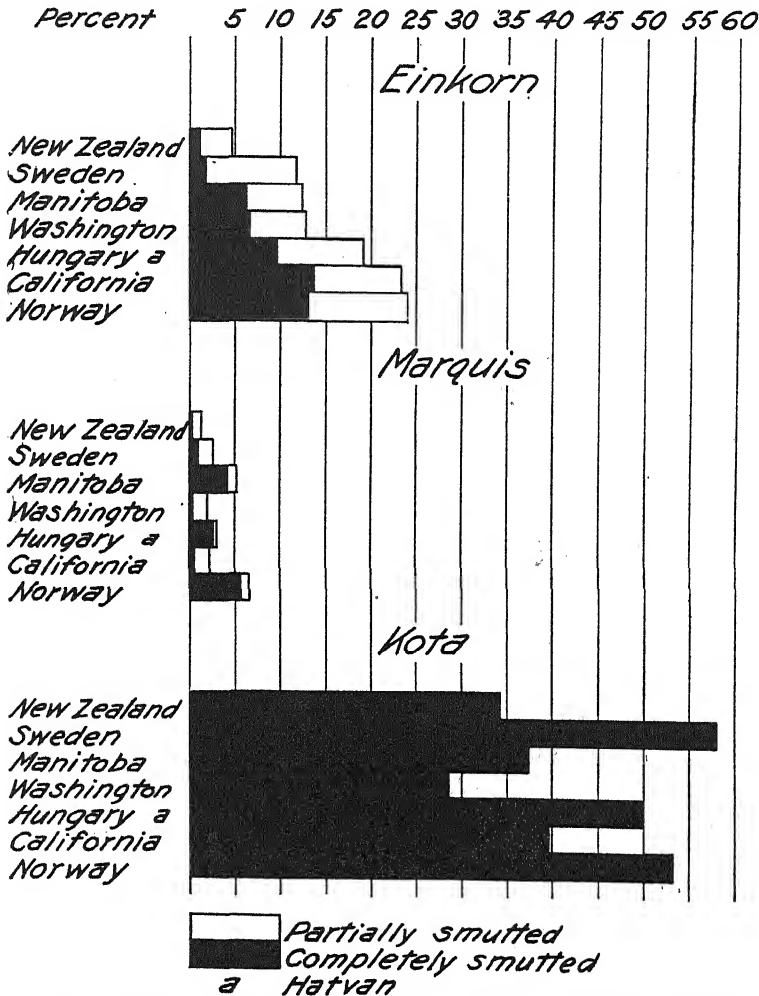


FIG. 18. The percentages of infection in Einkorn, Marquis, and Kota inoculated with seven collections of *Tilletia tritici* in 1926.

TABLE 14.—The percentage of smutted heads in Marquis and Kota wheats and in Einkorn inoculated with seven collections of *Tilletia tritici* at University Farm, St. Paul, Minn., in 1926

Source of inoculum	Germination of chlamydo-spores in per cent	Percentage of smutted heads						
		Einkorn			Marquis			Kota
		Partial	Complete	Total	Partial	Complete	Total	
New Zealand..	80	3.7 ^b	1.1	4.8	1.0	0.3	1.3	34.5
Hungary ^a	65	9.5 ^c	9.7	19.2	0.4	2.7	3.1	50.4
Norway	70	11.1	13.2	24.3	0.6	5.9	6.5	53.4
Sweden	80	9.9	1.9	11.8	1.8 ^b	0.7	2.5	58.0
Manitoba	80	5.8	6.4	12.2	1.0	4.2	5.2	37.5
California	80	10.0	13.7	23.7	1.5	0.5	2.0	40.0
Washington	65	4.0	6.5	10.5	1.4	0.4	1.8	28.5
Uninoculated	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0

^a Hatvan.

^b Individual heads lightly smutted.

^c Individual heads almost completely smutted.

surance that they will be resistant in other geographical areas. Einkorn, C. I. 2433, for example, is comparatively resistant to form 1 of *T. tritici* from New Zealand, but is susceptible to form 2 from Norway. Gaines (20) also recently found that varieties which were resistant to bunt in one geographical area are not necessarily resistant in another. *T. tritici* from Germany was found to have different pathogenic capabilities from that which is common in eastern Washington. The American wheats were susceptible to the German form while the German wheats succumbed more readily to the American form.

There is, furthermore, no assurance that resistant varieties of wheat which have been developed for a particular locality will remain resistant over a long period of time. In the hard red spring wheat region Marquis and the durum wheats have been resistant to bunt prior to 1924. In subsequent seasons bunt has continued to be epidemic in the durums, so one may conclude that this outbreak has not been due to unusually favorable environmental conditions but rather to the occurrence of a new physiologic form in the region. Stephens (47) reported a similar occurrence of a new physiologic form of bunt in Oregon. Heretofore immune and highly resistant varieties of wheats, such as White Odessa, Martin, and Albit, suddenly became smutted in 1927. Since varieties of wheat resistant to bunt in one geographical area may not be resistant in another area, and since new forms are appearing in certain localities, it is evident that a study of physi-

TABLE 15.—*The percentage of smutted heads in Marquis, Kota, and Pentad wheats and in Einkorn inoculated with seven collections of Tilletia tritici at University Farm, St. Paul, Minn., in 1927*

Source of inoculum	Germination of chlamydo-spores in per cent	Percentage of smutted heads											
		Einkorn			Marquis			Kota			Pentad		
		Partial	Complete	Total	Partial	Complete	Total	Partial	Complete	Total	Partial	Complete	Total
New Zealand	60	21.9	1.5	23.4	1.3	0.2	1.5	5.6	4.1	9.7	6.8	0.4	7.2
Hungary ^a	65	48.4	3.3	51.7	3.6	0.3	3.9	8.7	23.1	31.8	7.8	0.9	8.7
Norway	75	50.8	6.7	57.5	7.5	5.8	13.3	4.9	25.0	29.9	6.3	3.9	10.2
Sweden	60	40.1	2.4	42.5	2.6	3.1	5.7	8.9	17.1	26.0	7.3	0.9	8.2
Manitoba	80	35.2	2.9	38.1	1.8	2.1	3.9	6.3	11.1	17.4	3.8	0.9	4.7
California	70	48.7	9.4	58.1	1.7	0.9	2.6	6.3	24.8	31.1	5.7	0.7	6.4
Washington	75	34.0	8.8	42.8	0.4	0.8	1.2	1.8	6.1	7.9	2.8	0.8	3.6
Uninoculated	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

^a Hatvan.

TABLE 16.—*The relative susceptibility of Einkorn, Marquis, and Kota to four collections of Tilletia levis and to three of T. tritici at University Farm, St. Paul, Minn., in 1926*

Source of inoculum	Relative susceptibility ^c			Form number
	Einkorn	Marquis	Kota	
<i>Tilletia levis</i>				
Hungary ^a	R	R	S	1
Hungary ^b	R	M R	S	1
Minnesota	M R	M R	S	2
Egypt	M R	V R	M S	3
<i>Tilletia tritici</i>				
New Zealand	V R	V R	M S	1
Norway	M R	M R	S	2

^a Hatvan.

^b Debreizen.

^c V R = very resistant; M R = moderately resistant; M S = moderately susceptible; S = susceptible.

ologic specialization as it affects the problem of breeding is of primary importance.

Pathogenicity of Physiologic forms of Ustilago hordei

Seven different physiologic forms of *U. hordei* were recognized in culture. It is important, of course, to know whether these forms differ in pathogenicity also. And particularly important is the question as to whether any of them are so virulent as to be able to infect varieties of barley that have been generally resistant. About 135 varieties and selections of barley were tested for their reaction to loose and covered smuts in 1924 to 1927. These varieties were heavily inoculated artificially with chlamydospores of *U. hordei*, but so little smut developed in most of them that the results were not considered significant. Observations were made also on the reaction of several varieties to the two smuts in other plots at University Farm, St. Paul, and at the University of Minnesota sub-stations located at Crookston, Grand Rapids, Morris, and Waseca. Lion, C. I. 923, which is very susceptible to loose smut, was found to be immune to covered smut. White Spring Hulless (C. I. not known) was found to be most susceptible to both the loose and covered smuts. Odessa, C. I. 182, Trebi, Minn. 448, Glabron, Minn. 445, and Svanhals, C. I. 187, may be classed as susceptible, while Manchuria, Minn. 184, and Svansota, Minn. 440, may be classed as resistant. The reaction of these varieties to smut was determined under Minnesota conditions. This does not mean, however, that they would

be resistant when grown in another geographical area. In fact Faris (16) distinguished four physiologic forms by differences in pathogenicity.

The writer made inoculations with two culturally distinct physiologic forms of *U. hordei* in order to determine if they could be differentiated on the basis of pathogenicity. In the spring of 1927 Lion barley, C. I. 923, Himalaya, C. I. 620, Manchuria, Minn. 184, Svansota, Minn. 440, Trebi, Minn. 448, and Wisconsin Pedigree, Minn. 454, were inoculated with sporidial cultures collected in Italy and at University Farm, St. Paul, Minn. The inoculum was grown in Erlenmeyer flasks on 2 per cent potato dextrose agar for a period of two weeks. Previous to inoculation the barley seed was treated with a 1:320 solution of formaldehyde, thoroughly washed in water and then dried. The seed was then allowed to germinate and inoculations made when the coleoptile had appeared by immersing the seed for a period of 20 hours in a suspension of sporidia in distilled water. All the seedlings were then sown on the same day in duplicated row rows.

The results obtained from the inoculation of six varieties of barley with two forms of smut are summarized in table 17. No smut developed in Manchuria, Svansota, Trebi, or Wisconsin Pedigree when inoculated with form 2 which was collected at St. Paul, Minn. There were, however, differences in the virulence of the two forms on Himalaya and Lion. Himalaya proved to be susceptible to form 2 and highly resistant to form 1. On the other hand, Lion was susceptible to form 1 and immune to form 2. It is probable that there are many more forms which differ pathogenically.

Only two of the seven cultural forms were tested, but, not only were they pathogenically distinct, but one of them, the form from Italy, infected Lion barley which has been immune to *U. hordei* in Minnesota up to the present time.

Varietal Resistance of Oats to Ustilago levis and Ustilago avenae

In connection with the study of physiologic forms of oat smuts, a study was made of the varietal resistance of species and varieties of *Avena* to *U. levis* and *U. avenae*. The tests extended over a period of four seasons, during which 58 varieties belonging to six species of *Avena* were grown. Reed (34) and Reed and Griffiths (36) have reviewed the work of other investigators up to 1925. Reed (34) tested 154 agronomic strains and varieties of oats at the University of Missouri, which, when further tested by Reed and Griffiths (36) at widely separated stations, behaved in about the same way as they had in Missouri. Of the seven host species studied, *Avena brevis* Roth. and *Avena strigosa* Schreb., developed only smut-free plants. *Avena sterilis* L. was highly resistant. The other species were generally susceptible, although two varieties, Black Mesdag and Fulghum, were immune from both smuts.

In the writer's tests, the seed of each variety was sown in single row rows at the rate of 15 grams to each row, this making about 300 plants to the row. Just before sowing, the seeds were uniformly inoculated with a mixture of chlamydospores of *U. levis* and *U. avenae*. Percentages of smut were obtained by counting the number of diseased heads instead of diseased plants. As the two smuts can be considered as one for practical purposes, all smutted heads were counted without distinguishing between the two kinds of smut.

The data on varietal resistance at University Farm are summarized in table 18. All of the averages in table 18 are not strictly comparable, because in a few instances varieties were not tested all four seasons, but they are valuable in that they do furnish an indication of resistance and susceptibility. With few exceptions the results obtained confirm those of previous investigators. *A. nuda* was found to be extremely susceptible, *A. strigosa* highly resistant, and *A. brevis* immune. With one exception the varieties of *A. sterilis* were resistant. In Iowa Burt (C. I. number not known) an average of 5.4 per cent of smut developed. Most of the varieties of *A. sativa* and *A. sativa orientalis* were susceptible. A few, however, were either immune or highly resistant. Black Mesdag, C. I. 1877, and Markton, C. I. 2053, were immune throughout the four seasons. Early Champion, C. I. 1930, was the most susceptible variety of this group, it having an average of 29.9 per cent of smut. Black Mesdag, which was found to be immune by other investigators, also was immune in the writer's tests. Golden Giant, C. I. 1606, was immune in all tests when inoculated with both smuts. In Reed's tests (34), however, this variety proved to be susceptible when inoculated with smut in Missouri. In this variety he obtained 16.0 per cent of *U. avenae* and 45.6 per cent of *U. levis*. This difference is undoubtedly due to the presence of a different physiologic form of the fungus in Missouri than the one prevalent at University Farm, St. Paul, Minn., as Reed (35), in a later publication, has shown that there are physiologic forms of the oat smuts which differ in their pathogenic capabilities.

Five physiologic forms of *U. levis* and eighteen of *U. avenae* were differentiated on the basis of cultural characteristics. An attempt was made to determine whether these forms differed also in their pathogenic capabilities and whether varieties of oats that had proven immune or highly resistant to smut at University Farm would be attacked by any of these physiologic forms. Ten varieties of oats were inoculated individually with sporidia of three physiologic forms of *U. levis* and five forms of *U. avenae* as described in similar tests with *U. hordei*. Unfortunately, however, no results were obtained, since smut failed to develop in any of the varieties. Evidently the technique was faulty, but in what respect has not yet been determined.

TABLE 17.—*Summary of data on tests for pathogenicity of two physiologic forms of Ustilago hordei at University Farm, St. Paul, Minn., in 1927*

Variety	Smut from Italy					Smut from St. Paul					Uninoculated
	Series A		Series B			Series A		Series B		Average per cent infected	
	Total no. heads	Per cent infected	Total no. heads	Per cent infected	Average per cent infected	Total no. heads	Per cent infected				
Lion	515	8.8	412	5.6	7.2	489	0.0	502	0.0	0.0	
Himalaya	390	Tr.	409	0.0	Tr.	246	21.5	311	12.5	17.0	
Manchuria	479	0.0	502	0.0	0.0	438	Tr.	461	0.0	Tr.	
Svensote	480	0.0	496	0.0	0.0	468	Tr.	492	0.0	Tr.	
Trebi	460	0.0	413	0.0	0.0	481	Tr.	399	0.0	Tr.	
Wisc. Ped.	417	0.0	391	0.0	0.0	397	Tr.	431	Tr.	Tr.	

TABLE 18.—*The percentage of smut (Ustilago avenae and U. levis) on oat varieties at University Farm, St. Paul, Minnesota, in 1924-1927*

Species and varieties	C. I. number	Year and percentage of smutted heads				
		1924	1925	1926	1927	Average
<i>Avena nuda</i>		68.0	—	—	16.2	42.1
<i>A. strigosa</i>		0.0	0.0	1.0	0.0	Tr.
<i>A. brevis</i>		0.0	0.0	0.0	0.0	0.0
<i>A. sterilis</i>						
Burt		0.0	1.1	0.0	0.0	Tr.
California Burt		1.5	1.2	3.9	1.4	2.0
Iowa Burt		5.0	5.0	7.8	3.8	5.4
King		0.0	1.0	Tr.	Tr.	Tr.
Red Rustproof	1356	0.0	2.9	0.0	Tr.	0.7
<i>A. sativa</i>						
Anthony (Minn. 686)...		2.0	1.3	8.3	11.0	5.8
Aurora	831	3.0	15.9	8.8	9.7	9.4
Awnless Probsteier	1888	1.0	8.7	4.5	7.8	5.5
Belyak	1630	0.0	0.6	1.5	5.0	1.8
Black Mesdag	1877	0.0	0.0	0.0	0.0	0.0
Black Norway		0.0	3.5	2.0	2.5	2.0
Canadian	1625	1.0	7.2	11.0	4.0	5.8
C. I. 602		1.0	3.0	5.5	11.4	5.2
C. I. 603		0.0	4.0	2.5	3.6	2.5
C. I. 606		1.0	0.4	Tr.	0.7	0.5
C. I. 620		1.0	16.5	17.0	6.8	10.1
Culberson	273	0.0	8.6	3.0	2.0	3.4
Danish Island	519	1.0	6.2	9.0	16.4	8.2
Early Champion	1930	8.0	54.6	30.5	26.6	29.9
Early Dakota		1.0	2.8	11.0	11.2	6.5
Early Gothland	1723	1.0	2.5	4.5	7.2	3.8
Garton 473	1613	1.0	3.0	3.5	1.4	2.2
Garton 691		5.0	0.0	5.0	1.8	3.0
Golden Drop	1890	0.0	7.2	6.3	8.4	5.5
Gopher	2027	0.5	23.2	8.0	3.8	8.9
Green Russian		1.0	1.5	4.5	2.4	2.4
Irish Victor	1896	1.0	Tr.	5.0	5.6	2.9
Japan Selection	1889	2.0	3.5	11.5	29.6	11.7
Joanette	1762	1.0	14.9	9.9	7.8	8.4
June	1902	3.0	11.4	9.0	8.6	8.0
Kanota	839	4.0	4.6	8.5	2.6	4.9
Kherson	459	0.0	24.8	20.0	13.2	14.5
Kherson Selection	1905	1.0	35.5	17.5	18.4	18.1
Lincoln	1463	1.0	18.2	6.0	7.4	8.2
Markton	2053	0.0	0.0	0.0	0.0	0.0
Minota	1285	1.5	20.2	18.0	11.0	12.7
Monarch	1682	0.0	6.2	2.5	4.6	3.3
Monarch Selection	1879	1.9	2.4	4.5	7.0	4.0
North Finnish	1882	0.0	3.6	8.0	8.0	4.9
Old Island Black	1881	0.0	17.6	9.8	7.4	8.7
Scottish Chief	1901	0.0	0.0	2.8	1.8	1.2
Silvermine	1629	0.0	0.0	0.5	5.4	1.5
Silvermine Selection	1894	0.0	0.0	1.5	4.8	1.6
Sixty Day	826	0.5	26.9	3.0	8.6	9.8
Sixty Day Selection	1906	0.0	10.6	0.5	2.8	3.5
Swedish Select	802	0.0	8.6	6.0	7.6	5.6
Tobolsk	1709	1.0	3.6	4.0	7.4	4.0
Victor	803	2.0	10.0	18.0	12.2	10.6

TABLE 18.—*Continued*

Species and varieties	C. I. number	Year and percentage of smutted heads				
		1924	1925	1926	1927	Average
<i>A. sativa orientalis</i>						
Black Tartarian		0.0	—	16.0	6.8	7.6
Garton 585	1868	0.0	0.6	1.0	1.0	0.7
Garton 748		0.0	0.0	6.0	13.4	4.9
Garton 784		5.0	6.0	11.0	3.2	6.3
Garton Gray		0.0	—	11.5	4.2	5.2
Green Mountain	1872	2.0	3.6	5.0	7.8	4.6
Golden Giant	1606	0.0	0.0	0.0	0.0	0.0
Sparrowbill	1604	3.0	1.5	11.5	3.0	4.8
Storm King	1602	3.0	1.8	8.0	0.6	3.4
White Tartar	1614	0.0	28.2	6.0	7.2	10.4

DISCUSSION AND CONCLUSIONS

There are many physiologic forms of the cereal smut fungi. They differ greatly in cultural characteristics, in physico-chemical relations and, in some cases at least, in pathogenicity. On culture media the physiologic forms differed strikingly from each other in the following characters: color, topography of colonies, character of surface, consistency, and type of margin. The cultural differences can be detected only when the proper differential materials are used. There may be decided differences between forms grown under one set of environmental conditions which may not be apparent when the same forms are grown in another environment. Under identical conditions, however, the cultural characteristics of given forms are remarkably constant. The differences in the cultural characteristics of some of the physiologic forms are so great that one might even consider them as different species. On the other hand, the cultural characteristics of forms of different species are sometimes strikingly similar.

Twelve distinct forms of *U. nuda* and fourteen of *U. tritici* were distinguished on culture media. At present these two smuts are considered as distinct species. It is significant, however, that some of the forms of *U. nuda* resemble some of *U. tritici* more closely than they do certain other forms of *U. nuda*. The chlamydospores of the two smuts are alike, neither produces sporidia, and the effect on their respective host plants is the same. It would seem, then, that *U. nuda* and *U. tritici* are themselves nothing more than physiologic forms. The principal difference between them is that *U. nuda* infects barley and *U. tritici* infects wheat. It is quite possible, however, that this specialization does not always occur. In fact Humphrey and Tapke (23) have shown that *U. tritici* can infect rye. The writer considers it probable that some of the forms of *U. nuda* probably will infect wheat, and some of *U. tritici* probably will infect barley.

It is possible also that *U. levis* and *U. avenae* are physiologic forms of the same species. Five physiologic forms of *U. levis* and eighteen forms of *U. avenae* were distinguished on culture media. Here again the differences between certain forms of the same species were greater than those between some of *U. levis* and *U. avenae*. These two smuts have the same life history, they apparently have the same host range, the spores are of the same size and shape, and both are light on one side; in most cases it is impossible to tell the two apart. Furthermore, Reed (34) found that various strains and varieties responded similarly to inoculation with the two smuts. At present the two species are differentiated on the basis of markings on the chlamydospores walls. However, in a microscopic examination of the chlamydospores from a single head supposedly infected with *U. avenae*, all gradations in degree of echinulate markings, from practically smooth to highly echinulate, may be found.

Not only do the physiologic forms of some of the smuts differ in general appearance on culture media but they react quite differently to temperature. Physiologic forms of *U. tritici*, *U. nuda*, and *U. hordei* were differentiated on the basis of differences in optimum temperature for growth, as measured by the size of colonies and also by the amount of radial growth attained at certain temperatures. The fact that physiologic forms of smuts do react differently to temperature might very easily account for the development of characteristic physiologic forms in certain geographical areas. Furthermore, if these forms which differ in their reaction to temperature also differ in their ability to attack certain varieties, it would account, in part at least, for the variation from year to year in the susceptibility of certain varieties to smut. Not only do the physiologic forms differ in general appearance on culture media and in their physico-chemical reaction but some also differ pathogenically.

Physiologic forms of *U. hordei* were found to differ in their ability to infect certain varieties of barley. One form was very virulent on a variety that had always been immune to covered smut in Minnesota. On the other hand, it was only weakly pathogenic on a variety which is susceptible to a form of *U. hordei* collected at University Farm, St. Paul, Minn.

In a study of the varietal resistance of spring wheats to bunt caused by *T. levis*, the durum wheats as a class were found to be more resistant than the common wheats. There were, however, wide differences within both classes, ranging from highly resistant varieties to those completely susceptible. Resistance then is not correlated with any particular morphologic type and it should be possible to breed varieties of wheat having desirable agronomic characteristics which are highly resistant to bunt. This problem is complicated, however, by the fact that there are physiologic forms of the pathogenes causing bunt. Three physiologic forms of *T. levis* and

two of *T. tritici* differed in their parasitic capabilities on several varieties of wheat. It seems likely that several more forms could have been distinguished had more differential hosts been used. One of the forms of *T. tritici* is characterized by being relatively virulent on all the varieties tested while another form is only weakly parasitic or moderately virulent on the same varieties. There also are distinct differences in the virulence of the physiologic forms of *T. levis*.

The present studies have shown that there are numerous physiologic forms in the cereal smut fungi and that they are widely distributed throughout different geographical areas. Some of these physiologic forms differ in their pathogenic capabilities, and varieties which are immune or highly resistant to smut in one locality may be susceptible in another. As a result of this condition, breeding for smut resistance becomes more complicated and requires a thorough study of the number, distribution, and pathogenic capabilities of the physiologic forms of the pathogene. New physiologic forms of the smut fungi probably are being formed constantly, either by hybridization or mutation. Stakman and Christensen (45) have shown that inoculation with two strains of opposite sex is necessary in order that corn may become infected with *U. zeae*. Hybridization takes place in or on the corn plant, which means that new combinations result. A new physiologic form may then be produced as a result of hybridization. Dickinson (11 and 12) has shown that when oat seedlings are inoculated with sporidia of *U. levis* of one gender, (A) or (B), infection will not take place. When, however, they are inoculated with hyphae resulting from the fusion of sporidia of two genders, (A) and (B), or from hyphae which result from a fusion between sporidia of *U. levis* and *U. hordei*, infection will take place. Recombination and segregation may occur here also, with the result that a new physiologic form may be formed. Christensen and Stakman (9) found mutations to occur frequently in *U. zeae*. These mutants differed from their parents not only in general appearance but also in pathogenicity. Just how the sectors of *U. hordei* and *U. avenae* arose can not be definitely stated, but the significant thing is that new physiologic forms are being formed in the cereal smut fungi. If they differ in their pathogenicity as do many of the forms which are already present, which is very likely, then breeding for smut resistance even as a regional problem becomes a continuous one.

SUMMARY

1. *Ustilago tritici*, *U. nuda*, *U. hordei*, *U. levis*, *U. avenae*, *Tilletia levis*, and *T. tritici* are group species consisting of many physiologic forms. The physiologic forms are widespread in the United States and foreign countries.

2. Fourteen physiologic forms of *U. tritici*, twelve of *U. nuda*, seven of *U. hordei*, five of *U. levis* and eighteen of *U. avenae* were studied in detail. The forms can be distinguished in culture by the following characters: color, topography, surface, consistency, and type of margin.

3. The differences between physiologic forms in culture sometimes are very great but they can be distinguished only when grown on the proper media. When grown under identical conditions the cultural characteristics of the physiologic forms are constant.

4. The differences in cultural characteristics of different forms of *U. nuda* are sometimes far greater than those between the so-called species *U. tritici* and *U. nuda*. The morphological differences between these two fungi are scarcely pronounced enough to consider them as species and they are considered by the writer as physiologic forms.

5. The differences in cultural characteristics of different forms of *U. levis* are sometimes far greater than those between the so-called species of *U. levis* and *U. avenae*. It is considered by the writer that these are possibly nothing but physiologic forms.

6. Some of the physiologic forms of *U. tritici*, *U. nuda*, and *U. hordei* differ also in their physico-chemical reactions. Differences were noted in the optimum temperatures for growth and in the amount of growth, measured by the diameter of the colonies, at different temperatures.

7. When subjected to different temperatures, physiologic forms of *U. nuda* respond differently with respect to the formation of counter-clockwise growth of mycelium.

8. Oat varieties varied greatly in their resistance to smut. Three varieties, Markton, Black Mesdag and Golden Giant, and *Avena brevis* were immune. *A. strigosa* and varieties of *A. sterilis* were in general resistant. Most of the varieties of *A. sativa* and *A. sativa orientalis* were susceptible.

9. Wheat varieties varied greatly in their resistance to bunt; none were immune. The durumms as a class are more resistant than the common types. The common wheats possess all gradations of resistance and susceptibility.

10. Two physiologic forms of *Ustilago hordei* can be recognized by their parasitic behavior on Lion and Himalaya barley. Lion is immune to the forms prevalent in Minnesota and susceptible to an Italian form. Himalaya is highly resistant to the same Italian form but susceptible to the Minnesota form.

11. *Tilletia levis* and *T. tritici* both comprise distinct physiologic forms which can be recognized by their degree of virulence on Kota, Marquis, and Pentad wheats and on Einkorn.

12. Collections of *T. levis* were obtained from Minnesota, Italy, Egypt, and two localities in Hungary. There were at least three physiologic forms

in these collections: one from Minnesota, one from Hungary, and one from Egypt.

13. Collections of *T. tritici* were obtained from New Zealand, Hungary, Norway, Sweden, Canada (Manitoba), and from Minnesota, California, and Washington in the United States. Two forms can be recognized readily: a virulent one from Norway and a relatively weak one from New Zealand.

14. It seems likely that a considerable number of forms both of *T. levis* and *T. tritici* can be distinguished if the proper differential hosts are used.

15. Sectors occurred in cultures of *U. hordei* and *U. avenae* and differed from the parents in color and rate of growth.

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ON THE OCCURRENCE OF PYCNIA AND AECIA IN CERTAIN RUST FUNGI¹

J. H. CRAIGIE

Evidence secured by experimentation in the greenhouse has demonstrated that *Puccinia helianthi* Schw. and *P. graminis* Pers. are heterothallic; that the majority, at least, of pustules derived from infections by single sporidia of these rusts never produce aecia;² and that when pycniospore-containing nectar of such pustules is transferred from one to another, so that a thorough mixing is effected, aecia appear in a few days.³ A few additional observations and experiments bearing on the problem of sex in rust fungi are set forth in this paper.

For the sake of convenience in what follows, pustules of monosporidial origin are designated as "simple" pustules, and those of bisporidial origin as "compound" pustules.

EVIDENCE OF HETEROTHALLISM IN PUCCINIA GRAMINIS

It was intimated in one of the previous papers³ that *Puccinia graminis* is heterothallic, but, as that paper treated specifically of the function of the pycnia of rusts, no data were furnished to show the relative numbers of compound pustules and simple pustules which did, and which did not, produce aecia. A statement concerning this point will first be made.

Both simple and compound pustules of *P. graminis* were obtained on young leaves of barberry (*Berberis vulgaris*) by sowing sparsely the sporidia of germinating teliospores over the surface of the leaves. Figure 1 shows a barberry leaf which was photographed 20 days after it was inoculated. A compound pustule bearing aecia appears on the left-hand side of the midrib. On the right-hand side are three simple pustules in which no aecia have developed. The pitted appearance of these three pustules is due to the formation of haploid mycelial wefts which develop in the simple pustules just underneath the epidermis, and simulate in general contour that of a young aecium. They never break through the epidermis, however, and rarely, if ever, of themselves produce aeciospores. This point will be discussed in a subsequent paper.

¹ Contribution from the Division of Botany, Dominion Experimental Farms Branch, Ottawa.

² Craigie, J. H. Experiments on Sex in Rust Fungi. *Nature* 120: 116-117. 1927.

³ Craigie, J. H. Discovery of the function of the pycnia of the rust fungi. *Nature* 120: 765-767. 1927.

The pustules first appeared as tiny dots. As soon as they became evident, the position on the leaf of each individual pustule, and of each pair of pustules which might be expected to coalesce later, was mapped on a label which was then attached to the leaf. In this way it was possible to tell, when the pustules became older, which pustules were simple, and which were compound. If the distance between a pair of pustules was greater than 4 mm., each of the components was considered as a simple pustule, as the two seldom achieved a thorough coalescence. Usually the points at which infections took place on the leaf were easily discernible, but sometimes two infections occurred so close together that it was extremely difficult to decide whether or not there were two infections. This was an ever-recurring difficulty. Where there was any doubt, the pustules were always classed as simple. For this reason certain pustules which were very probably compound were tabulated as simple pustules.

As the number of barberry plants available and suitable at any one time for inoculation purposes was limited, the inoculating was done at different

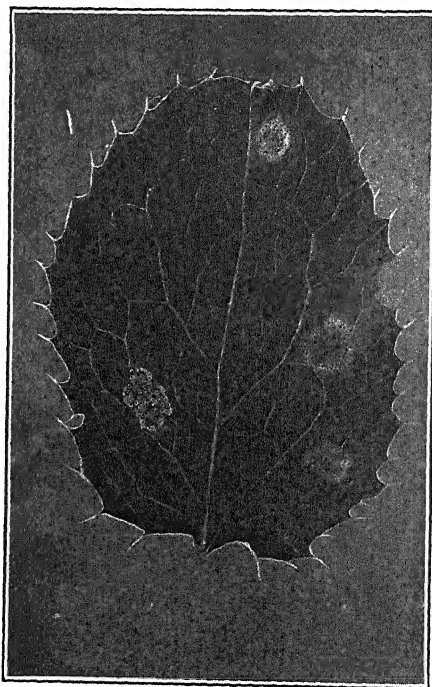


FIG. 1. Under side of a barberry leaf showing one compound pustule with aecia, on left of midrib, and three simple pustules without aecia, on right of midrib.
Photographed 20 days after inoculation. $\times 2$.

times. The data were recorded 20 days after each inoculation. Before the end of that time, the majority of the paired pustules, designated as compound, had completely coalesced, and where the paired pustules, or components, were of opposite sex, aecia had appeared. In a few of the compound pustules in which the components were relatively far apart, coalescence was not yet thoroughly accomplished. The results are summarized in table 1.

TABLE 1.—*Number of simple and compound pustules of Puccinia graminis on leaves of Berberis vulgaris in which aecia had developed and in which aecia had not developed 20 days after inoculation*

Kind of pustule	Total no. of pustules	No. of pustules	
		with aecia	without aecia
Simple (monosporidial in origin)	174	11	163
Compound (bisporidial in origin)	59	24	35

It should be mentioned that these data were collected before the function of the pycnia was discovered, and it is quite possible that, in at least some of the 11 simple pustules which gave rise to aecia, the development was induced by the transfer to them of nectar from pustules of opposite sex, by insects or other agencies. It is possible also that a few others, although tabulated as simple pustules, were really bisporidial in origin.

From time to time after the data recorded in table 1 were taken, some pustules of both types, which had hitherto produced no aecia, developed aecia. The appearance of aecia in a few of these compound pustules may be explained by the fact that the two components of each pustule, although of opposite sex, were at first rather widely separated (about 4 mm.), and consequently the interaction of their mycelia was delayed. The belated development of aecia which occurred in the other compound pustules and in the simple pustules was probably induced, at least to some extent, by a fortuitous transference to them of nectar from pustules of opposite sex. In some of the pustules aecia may have arisen spontaneously, but doubt is thrown on this possibility by the results obtained with simple pustules of *P. helianthi* recorded elsewhere in this paper.

Many of the pustules, both simple and compound, which did not produce aecia persisted for eight or nine weeks, some even for a longer time. When the experiment finally terminated, it was found that of the 174 simple pustules and the 59 compound pustules, 117 of the former and 23 of the latter had failed to develop aecia, a result which, after due allowance is made for the imperfections of the experiment, is fairly conclusive evidence of the heterothallic nature of this rust.

AECIA IN SIMPLE PUSTULES

When the effect of mixing the nectar of simple pustules of opposite sex was discovered, suspicion arose concerning the ability of such pustules to produce aecia spontaneously. In the early stages of the investigation, approximately 40 per cent of these pustules developed aecia, but later, when the plants bearing the pustules were kept in cages, aecia appeared in a very much smaller number. The inference was drawn that quite possibly, in the frequent examination of these pustules—the leaves were turned over by thumb and fore-finger, the nectar of (-) pustules was occasionally carried to (+) pustules, and *vice versa*, thus inducing the development of aecia in some pustules. Moreover, no protection was afforded these pustules against the visitation of flies or other insects which might by chance have entered the greenhouse.

In order to get more exact information as to whether simple pustules were capable in themselves of producing aecia, the following experiment was undertaken. Leaves of sunflower seedlings growing in the greenhouse were inoculated with sporidia of *Puccinia helianthi* when the two first true leaves were about one inch or slightly less in length. The sporidia were allowed to fall sparsely on to the upper surface of the leaves.

Usually about six seedlings grew in a pot, but, whenever the minute pustules appeared, each plant showing infection was transplanted alone in a separate pot and covered with a screen wire cage. It was thought that by keeping each plant in an individual cage the opportunity for the transfer of nectar by any insect which might enter the cage would be reduced to a minimum. In general, not more than one pustule occurred on any individual leaf. Indeed, many of the plants bore but a single pustule. Infrequently, however, two or more occurred on the same leaf. All uninfected leaves were removed from the plants and no new ones were allowed to develop.

With the exception of a few compound pustules which were readily discernible, and a small number of pustules which were marked as simple but which produced aecia within 12 or 14 days, all the others were apparently simple. Those which were at first marked as simple but developed aecia within two weeks from the time of inoculation possibly arose from two infections which took place so close together that the two infections appeared as one. Sometimes it was difficult to determine with certainty whether a single sporidium caused the infection, or whether it arose from two sporidia which alighted side by side on the leaf. It can not be said with confidence that these apparently simple pustules were in reality compound, but the likelihood is that they were. In order to give any compound pustules which might have had components of opposite sex an opportunity

to develop aecia and to avoid dealing with doubtful cases, no data were recorded until the pustules were 17 days old. There were altogether 228 pustules, all apparently simple ones, in which no aecia at all were evident. Of these, 93 were borne singly, one on each plant; of the remaining 135, usually two, but sometimes three or even four, occurred on the same plant. None of the pustules which developed on the cotyledons were considered, for the majority of the cotyledons became chlorotic and died comparatively early in the experiment.

Not all of the pustules persisted for the same length of time. Some became necrotic during the fourth week; most of them died before the end of the sixth week; but a few were still living and exuding nectar in their peripheral region when seven weeks old. The experiment terminated at the end of the seventh week. Within that time, aecia appeared in 11 of the 228 pustules. There was no regularity in their time of appearance, as if in response to a common stimulus. First one pustule—then a few days or a week later another pustule — would give rise to aecia, as if influenced by a separate and distinct stimulus. The results are summarized in table 2.

TABLE 2.—*Number of simple pustules of Puccinia helianthi, over 17 days old, which produced aecia and which did not produce aecia*

Pustules per plant	No. of pustules which produced	
	Aecia	No aecia
One	2	91
More than one	9	126
Total	11	217

From the time the pustules first appeared, an examination of each plant was made daily in order to destroy any insect which might have found its way through the mesh of the cages and thus gained access to the plant. White flies (*Aleyrodes*) and thrips (*Heliothrips*) were about the only ones which succeeded in doing this. Any of these that were found were removed and destroyed. The former were rarely ever seen in contact with the pustules, but the latter showed a decided preference for nectar as a diet. In spite, therefore, of considerable precautions, there was the possibility, especially in those cages in which the plants bore more than one pustule, that, during the seven weeks over which the experiment extended, some slight mixing of nectar took place.

Possibly, with the absolute exclusion of insects, or other means by which nectar might be transferred from one pustule to another, aecia would have arisen in none of these pustules. This, however, was not demonstrated.

At present, it is impossible to say that aecia do not arise spontaneously in pustules of monosporidial origin, but it has been clearly shown that, under the conditions of the experiment, only a very small percentage of such pustules did give rise to aecia. It is desirable that this point should be definitely settled, but to do so would require very rigid control and many more pustules than the writer can hope to have available this year.

OCURRENCE UNDER NATURAL CONDITIONS OF PYCNIA UNACCOMPANIED
BY AECIA

So far as the writer is aware, it is not recorded that pustules of monosporidial origin occur in nature, although undoubtedly they have been seen by other observers and mistaken by them for young pustules in which aecia had not yet developed.

From observations made during the summer of 1927 and of 1928, it has been found that in nature pycnia are frequently unaccompanied by aecia in the following rusts: *Puccinia graminis*, on *Berberis vulgaris* var. *purpurea*; *P. coronata* Cda., on *Rhamnus cathartica*; *P. pringsheimiana* Kleb., on *Ribes grossularia* (cultivated and wild); and a *Gymnosporangium* sp. (possibly *corniculans* Kern.), on *Amelanchier alnifolia*. Very probably the phenomenon occurs under natural conditions in other rusts also, but, as those mentioned were the most accessible, observations were confined mostly to them.

Leaves of these hosts bearing young and apparently simple pustules were marked by means of a small tag as soon as the pustules were noticed. When the pustules were about 14 or 15 days old, those which showed no evidence at all of aecia were selected for further observation. It was thought that if any of the first marked pustules originated from two contiguous infections and the mycelia of the two were of opposite sex, aecia would appear in such compound pustules within this time; if the mycelia were of the same sex, no aecia would be expected. In all the carefully examined pustules, pycnia were invariably present.

From time to time during the next three or four weeks, these pustules were examined. Within that time, some of the pustules produced aecia, but others did not. All of the pustules which produced aecia and most of those that produced none became necrotic, but a few of the latter persisted fresh-looking and vigorous for a week or more longer. The results of these observations for 1927 are given in table 3.

It should be noted that none of these pustules were protected in any way from the visitation of insects, and there is little doubt that through their agency the transfer of nectar from one pustule to another took place, as insects of various kinds were seen flying about or crawling over the leaves

TABLE 3.—Summary of observations made in 1927 on the occurrence in nature of aecia in simple pustules

Name of rust	No. of pustules 14 days old	Observation period (weeks)	No. of pustules at end of period	
			with aecia	without aecia
<i>Puccinia graminis</i>	50	4½	37	13
<i>P. coronata</i>	61	2½	45	15
<i>P. pringsheimiana</i>	60	3	16	44
<i>Gymnosporangium</i> sp.	60	3	52	8

of the host plants, some even in contact with the pustules. However, it should also be noted that, on account of the excessive precipitation, the pustules were frequently washed, and only very seldom was there any noticeable amount of nectar available for transfer. This fact accounts, perhaps, for the failure of so many of the pustules to produce aecia, despite the activity of the insects.

During the summer of 1928, young pustules which were apparently monosporous in origin were again marked, but instead of leaving them exposed, as was done in the previous year, most of the leaves bearing them were covered with one ply of white cheese-cloth, in order to intercept as far as possible the visitation of insects. This protection excluded fairly effectively most of the winged insects, but not so well, perhaps, ants and small spiders.

Not all the pustules marked were found as soon as they appeared. At the time the coverings were applied, some would be at least one week old; most of them, however, were just appearing, or not more than two or three days old. Unfortunately, the pustules available for study were much less numerous than one could have wished. Practically no infection occurred on *Rhamnus cathartica* at the Agricultural College, Winnipeg. None at all were found on *Amelanchier alnifolia*. Only a few appeared on *Berberis vulgaris*, and the pustules on *Ribes grossularia* were relatively scarce.

Certain of the pustules on *Ribes* were left unprotected in order to serve as a check for the protected ones, and for comparison with the data collected the previous year. The results are summarized in table 4.

It will be seen from tables 3 and 4 that a much smaller percentage of the covered pustules in 1928 produced aecia than of the uncovered ones in 1927; and that, in 1928, less than 20 per cent of the covered pustules of *P. pringsheimiana* developed aecia compared with 50 per cent of the ones which were not covered. Apparently, by preventing the free access of insects to the pustules, the opportunities for transferring the nectar of one

TABLE 4.—Summary of observations made in 1928 on the occurrence in nature of aecia in simple pustules

Name of rust	No. of pustules	Observation period (weeks)	No. of pustules at end of period	
			with aecia	without aecia
<i>Puccinia graminis</i> (covered)	20	3	4	16
<i>P. coronata</i> (do)	2	3	0	2
<i>P. pringsheimiana</i> (do)	33	3	6	27
do (uncovered)	18	3	6	12

pustule to another were reduced, and, consequently, the number of simple pustules which remained free of aecia was augmented. It is not probable that the cheese-cloth covering intercepted sufficient sunlight to inhibit or retard materially the formation of aecia. This supposition is supported by the fact that a certain number of the covered ones produced aecia. Very probably the development of aecia in these pustules was induced by the

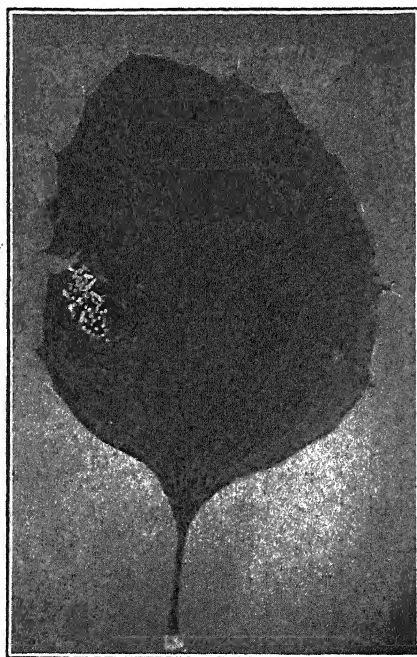


FIG. 2. Under side of a barberry leaf showing aecia present on the left-hand side, but not on the right-hand side, of a pustule of *P. graminis* which arose from an infection by natural inoculation. $\times 2$.

transfer of nectar by insects before the covering was applied. This of course is but conjecture.

From the fact that simple pustules of *P. coronata*, of *P. pringsheimiana*, and of the *Gymnosporangium* sp. behave under natural conditions as do similar pustules of *P. graminis*, and as *P. graminis* has been shown by experiments in the greenhouse to be heterothallic, it may be inferred that these three rusts also are heterothallic.

While the data recorded above were being collected, it was noticed that in a few of the pustules which had not previously developed aecia, aecia began to appear at one side or in one section of a pustule. This phenomenon was observed in simple pustules of *P. coronata*, of *P. pringsheimiana*, and of *P. graminis* (Fig. 2). A plausible explanation of this behavior is that the nectar from pustules of opposite sex had been deposited on the upper surface of those sections only of the pustules, and as a result aecia had developed.

Using this interpretation as a cue, an attempt was made to reproduce similar results in the greenhouse in simple pustules of *P. helianthi*. The nectar from a dozen or more pustules of this rust was collected and deposited in one drop. By so doing it was hoped that some nectar would be gathered

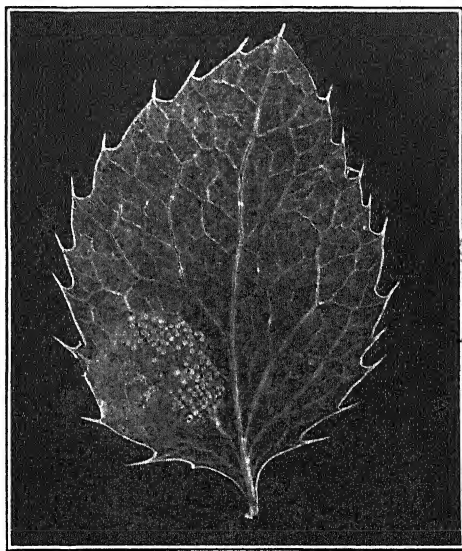


FIG. 3. Under side of a barberry leaf which was artificially inoculated in the greenhouse, showing aecia present on the right-hand side, but not on the left-hand side, of a simple pustule of *P. graminis*. When the pustule was 20 days old, a small amount of composite nectar was applied to the upper surface of that section which now bears the aecia. The photograph was taken 7 days after the nectar was applied. $\times 2\frac{1}{2}$.

from pustules of opposite sex and that the drop would contain both (+) and (-) pycniospores. Small amounts of this composite nectar were applied to one side only of the simple pustules when they were approximately four weeks old. In each case, aecia developed within four or five days on the under surface of the pustule directly beneath the section to which the nectar was applied. The experiment was repeated with simple pustules of *P. graminis*, three and one half weeks old, with similar results (Fig. 3).

The exudation of nectar is much more copious in pustules of *P. graminis* than in pustules of *P. helianthi*. Therefore, before the composite nectar was applied to any pustule of *P. graminis*, as much as possible of the nectar which it had itself produced was drawn off by means of a capillary tube. In most of the pustules the aecia remained localized, particularly in those of *P. helianthi*; but in some pustules of *P. graminis* there appeared to be a tendency for aecia, after they had formed in the treated section, to develop belatedly in the other part of the pustule—the course of development being from the treated section toward the opposite edge.

It is difficult to say to just what cause this belated formation of aecia was due. Possibly, in the course of a few days the pycniospores were carried slowly across the surfaces of the pustules by flowing movements of the nectar, owing to the fact that the pustules are usually convex or concave, rarely flat, and day by day as more nectar accumulated, adjustments in the level of the nectar took place, thus bringing the composite nectar into contact with pycnia in the untreated section of the pustule. This explanation seems to be supported by the fact that in pustules of *P. helianthi*, where, under greenhouse conditions, the amount of nectar exuded is comparatively small, aecial development was confined almost entirely to the sections treated. Another possible explanation for the development of belated aecia in the untreated sections of these pustules is that diploid mycelia may have gradually extended from the treated into the untreated parts and there given rise to aecia.

The occurrence in nature of aecia in one sector, or one part, of a simple pustule and the duplication of a similar phenomenon by experimental methods in the greenhouse are of interest in themselves, but they are mentioned chiefly to lend additional support to the evidence already given that *P. coronata* and *P. pringsheimiana* are heterothallic. In appearance and behavior they simulate the two rusts which have been shown by experimentation to be heterothallic.

SUMMARY

1. Experiments carried out in the greenhouse have shown that *Puccinia graminis* is heterothallic.

2. Experimentation has shown that, under greenhouse conditions, aecia develop spontaneously in few, if any, simple pustules of *Puccinia helianthi*.

3. Observations have shown that simple pustules of *Puccinia coronata*, of *P. pringsheimiana*, of a *Gymnosporangium* sp., and of *P. graminis* occur under natural conditions on their respective aecial hosts. This fact indicates that, in addition to *P. helianthi* and *P. graminis*, these three other rusts also are heterothallic.

4. Pustules of *P. coronata* and *P. pringsheimiana*, as well as of *P. graminis*, have been found in nature bearing aecia in only one sector, or part, of each pustule. Experimentation in the greenhouse with simple pustules of *P. graminis* and *P. helianthi* has reproduced similar conditions, a result which further indicates that *P. coronata* and *P. pringsheimiana* are heterothallic.

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A SIMPLE APPARATUS FOR ISOLATING SINGLE SPORES¹

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The securing of monosporous cultures of fungi has become a very important preliminary part of many mycological and phytopathological investigations. Several useful methods of isolating single spores have been described. Very few of these methods have come into general use, either because they can be employed only under certain conditions, or because of the high degree of technical skill required in their use. Elaborate micro-manipulators designed for even the most delicate work may be obtained, but they are rather expensive and are not to be found in most biological laboratories. Any efficient and simple apparatus which may be constructed in the laboratory, therefore, should be of general interest.

In a recent paper, Dickinson² described a method of isolating individual spores and bacteria. According to this method, a thin film of agar is placed on the surface of a cover-glass and the spores to be isolated are applied to this agar film. The cover-glass is then inverted over a Van Tieghem cell, and the latter placed on a glass slide under the objective of the microscope. Through an opening in the front of the Van Tieghem cell is inserted the arm of the "isolator," to the end of which a glass needle is attached, so that its point is directed upward to the surface of the agar. By means of a suitable mechanical device, the point of the needle may be moved up to any spot on the surface of the agar. When a particular spore is to be separated from its fellows, the tip of the needle is brought upward until it comes in contact with the film of moisture on the surface of the agar, immediately below the spot where the spore is resting. At this point, a cone of liquid is formed between the tip of the needle and the surface of the agar. By a lateral movement of the needle, the spore is carried along in the cone of liquid to the margin of the film of agar. When the needle is lowered, the cone of liquid is broken, and the spore is thus left on the surface of the agar at a convenient distance from all other spores. The point where the spore lies is now marked. Later, the spore and the agar about it may be removed by means of a sterile instrument and transferred to fresh medium.

Dickinson has also suggested that, with a needle of suitable size, the spore may be removed on the point of the needle when the cone of liquid is

¹ Published with the Approval of the Director, No. 798 of the Journal Series of the Minnesota Agricultural Experiment Station.

² Dickinson, S. A method of isolating and handling individual spores and bacteria. *Proc. Roy. Soc. Medicine* 19: 1-4. 1926.

broken. In practice, I have found that this method of removing individual sporidia from the promycelia of *Ustilago zaeae* and *Sorosporium reilianum* is highly satisfactory. Without question, the method may be employed in isolating single spores of almost all species of fungi. The apparatus to be described was used in isolating sporidia by this method. It serves the purpose quite as well as an expensive manipulator and possesses the advantage of being easily constructed from materials present in many laboratories.

Figure 1 shows the apparatus as it appears when attached to the stage

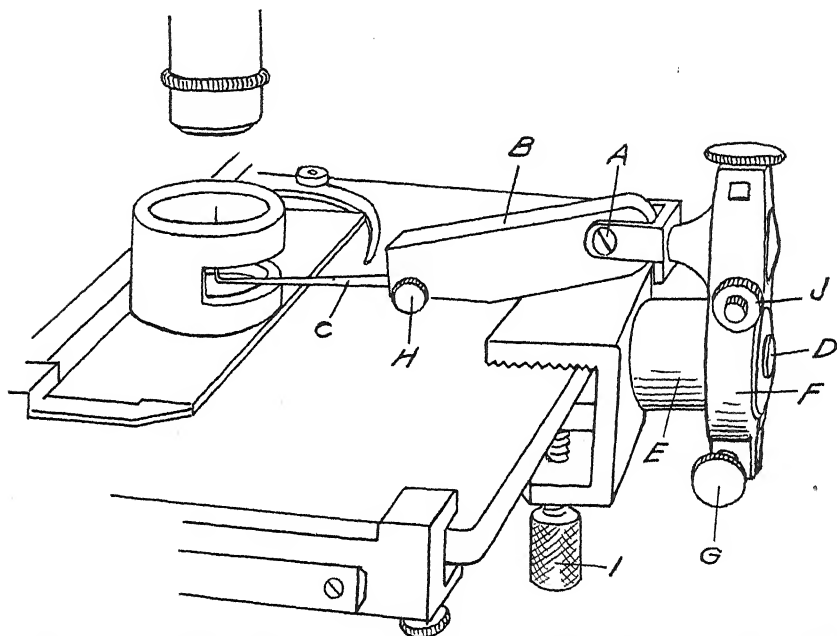


FIG. 1. Apparatus attached to the stage of the microscope. A, screw holding wooden arm in position. B, wooden arm bearing glass needle C. D, metal rod projecting from clamp, over which cylindrical piece of wood E is fitted. F, clamping ring held in place by the screw G. H, set screw holding glass needle in position. I, screw attaching the clamp to the microscope stage. J, screw for raising and lowering the needle point.

of the microscope. The device for raising and lowering the glass needle was obtained from a camera lucida manufactured by the Spencer Lens Co. When used with the camera lucida, this device is required to center the small prism above the ocular of the microscope. By removing the screw A, the prism may be replaced by an arm B made of hard wood, which serves to hold the glass needle C in position. The device was firmly attached to the stage of the microscope by means of a clamp. The clamp shown in the diagram was obtained from a microtome. From the side of the clamp there

projected a metal rod D. A cylindrical piece of wood E was fitted over this rod. The clamping ring F of the camera lucida, which would ordinarily be attached to the draw tube of the microscope, was then passed over the block of wood and secured in the desired position by the screw G.

Glass needles of a suitable size may be made from tubing about 3 mm. in diameter. The tube should be drawn out and bent to a right angle before the point is finished. When a satisfactory point has been obtained, the tube is cut off and the end inserted in the tip of the wooden arm, where it is held in position by means of the set screw H.

A Van Tieghem cell of the size to match the cover-glasses which are to be used may be cut from a block of paraffin. In the front of the cell, there should be an opening large enough to admit the needle and to permit of sufficient up and down movement. In practice, a cell about 25 mm. high has been found to be very satisfactory. The needle is adjusted by slightly releasing screws G and I and moving the apparatus until the point of the needle is exactly in the center of the field. When once this adjustment has been made, the position of the apparatus will require no further altering. All up and down movements of the needle are made by rotating screw J, while lateral movement is secured by shifting the position of the paraffin cell by means of the mechanical stage. Figure 2 shows the apparatus attached to a microscope.

The following procedure was adopted in removing from the promycelium the individual sporidia of *Ustilago zaeae* and *Sorosporium reilianum*: A number of petri dishes are fitted with glass Van Tieghem cells as described by Duggar³ (Fig. 3), each cell being provided with a cover-glass. A filter-paper with holes cut in it for the Van Tieghem cells is placed in each petri dish. When the dishes have been sterilized in a hot-air oven, a drop of sterile 1 per cent malt agar is placed on the lower side of each cover-glass. Using the dry needle method,⁴ a single chlamydospore is then placed on each drop of agar. When a spore has germinated and the sporidia have reached full size, the cover-glass is placed on the paraffin cell shown in figure 1. The spore is brought into the center of the field by moving the mechanical stage. By rotating screw J, the needle is moved upward until its point touches the liquid film immediately below the desired sporidium. The needle is lowered slightly, and then, by moving the mechanical stage, the sporidium in the cone of liquid is drawn away until it is some distance from the chlamydospore. After waiting a moment, the needle is lowered, when it will be found that the sporidium has left the agar and has remained

³ Duggar, B. M. *Fungous Diseases of Plants*. 508 pp. Ginn and Co. Boston. 1909.

⁴ Hanna, W. F. The dry needle method of making monosporous cultures of Hymenomycetes and other fungi. *Ann. Bot.* 38: 791-795. 1924.

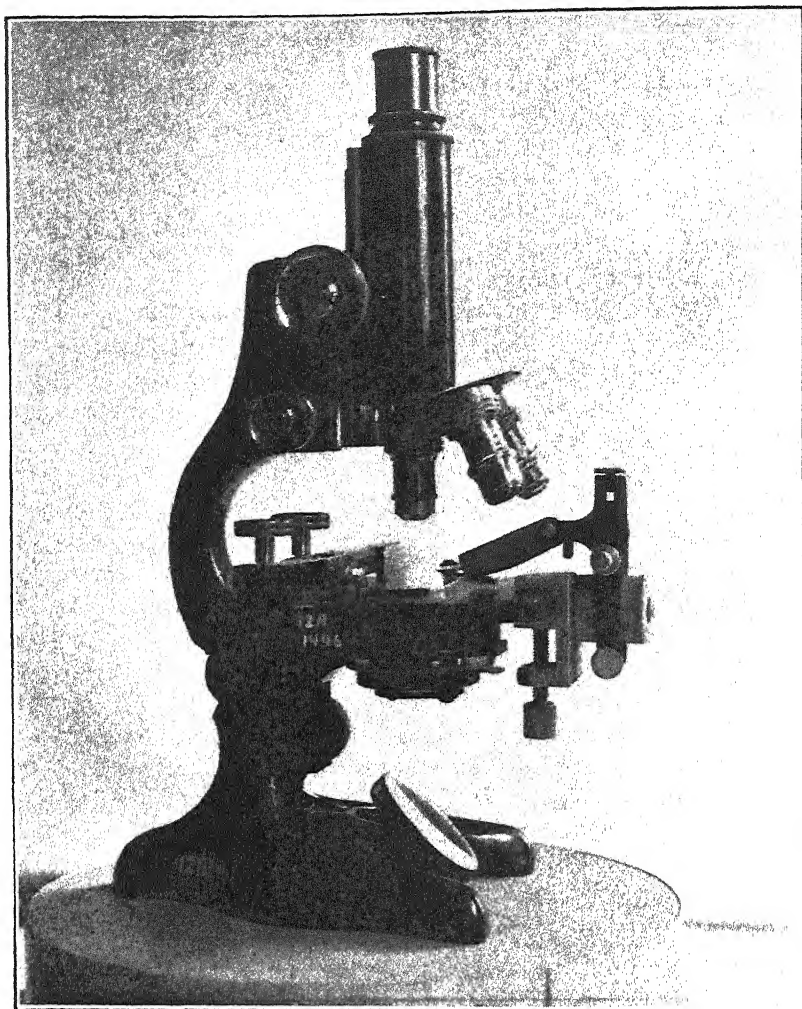


FIG. 2. Microscope equipped with apparatus for isolating single spores.

on the point of the needle. The cover-glass is removed, and another one having a drop of sterile malt agar is placed on the paraffin cell. The point of the needle is then brought upward until the sporidium is deposited on the surface of the agar drop. In this manner all of the sporidia may be removed from the promycelium.

The ease with which the sporidium may be removed from the agar drop is dependent upon the diameter of the needle point and the thickness of the liquid film on the surface of the agar. The primary sporidia of *U. zae*, when they have reached maturity, are about $8 \times 2 \mu$, and, in practice, it has

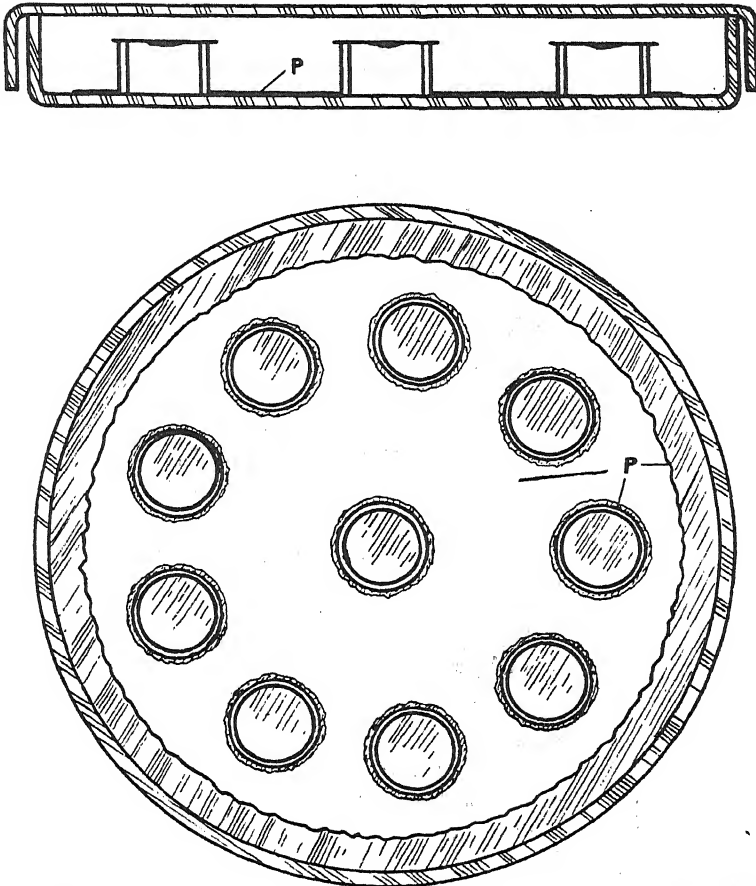


FIG. 3. Petri dish fitted with filter-paper, P, and ring cells for spore germination.

been found that they may be removed readily with a needle having a point about $15\ \mu$ in diameter. When the cover-glass is taken from the saturated atmosphere of the petri dish, the film of liquid on the surface of the agar is generally so thick that it is very difficult to remove the sporidium on the point of the needle. However, if the cover-glass is placed on the paraffin cell a few minutes before the work of isolation is to be commenced, the excess moisture will evaporate off and no difficulty will be experienced. If the air of the room is hot and dry, the moisture may evaporate from the drop too rapidly. This difficulty may be overcome by lining the inside of the paraffin cell with a strip of filter-paper and moistening the latter with sterile distilled water.

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BOOK REVIEWS

A List of Japanese Fungi Hitherto Known. By M. Shirai and K. Hara. Third, revised and enlarged, edition. vi + 448 + 14 + 30 + 12 pp. Nov. 1927, Shizuoka, Japan.

The first edition of this book was written in 1905 by Dr. M. Shirai, honorary professor of the Tokyo Imperial University. In 1917 it was revised and enlarged by Professor I. Miyake of the Tokyo Agricultural College. The present publication is the third edition, revised and enlarged by Mr. K. Hara, expert of the Prefectural Agricultural Society of Shizuoka. It is a very laborious and celebrated work, containing about 4,500 species of fungi hitherto known in Japan.

In addition, a list of bacterial plant pathogens known in Japan is also given by Dr. K. Nakata, professor of plant pathology of the Kyûsû Imperial University.

In this list the fungi are arranged alphabetically according to their Latin names, and some important references in European languages are given, although the host names are given only in Japanese. Therefore it seems to be very useful to the European and American mycologist as well as plant pathologist. This book may be secured from the reviser, Mr. K. Hara, c/o The Agricultural Society of Shizuoka Prefecture, Shizuoka, Japan. The price is three dollars, including postage.

DR. Y. NISIKADO, Ohara Institute, Kurashiki, Japan.

Plantsiektes Hul Oorsaak en Bestryding. By P. A. van der Bijl, M.A., D.Sc., Professor at the University of Stellenbosch, South Africa. 404 pp. Published by Nasionale Pers Beperk. Kaapstad, South Africa. 1928.

Professor van der Bijl's new text-book on plant pathology is written in an easily understandable way, so that not only the South African student but also the South African farmer will be able to profit by it.

The introduction gives a short summary of the history of plant pathology. The book proper is divided into three parts. In the first part are discussed the nature of the plant pathogenes, mainly the fungi; the characteristic structures of the main types of fungi and their terminology; and finally the main orders, families, and genera of fungi. The second part deals with the etiology and control of diseases of plants; and with the fungicides, their composition, usefulness, and application. The third part, which comprises half of the book, is devoted to the more important diseases of economic plants in South Africa. Each disease is treated in a brief way as follows: the name of the disease and the host, the conditions most favorable for the development of the disease, a short description of the fungus, its life history, and the means of control.

A large number of illustrations and references and an adequate index add to the usefulness of the book.

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PHYTOPATHOLOGICAL NOTES

A preliminary report¹ on mechanical transmission of the mosaic of Lilium auratum.—One of the chief troubles connected with the successful growing of *Lilium auratum* Lindl. in gardens and in greenhouses is a virus disease of the mosaic type. Its symptoms are a marked stunting of the susceptible together with a pronounced mottling of the leaves. Frequently, in cases of severe infection, the leaves are twisted and curled as well as mottled and the buds and flowers so distorted as to be valueless. Affected plants rarely survive more than a year or two in the garden.

Attempts were made to transmit this disease mechanically. The plants used were imported as bulbs from Japan and were grown in 6-inch pots in a greenhouse. Only normal, healthy plants which exhibited no mottling were used, and care was taken to keep the greenhouse free from insects. Twenty of these plants were kept as checks during the time the experiments were in progress. All check plants remained healthy.

The first successful transfer was obtained by inarching a healthy and a mosaic plant. The lower leaves were removed, and by means of a razor a small slice was taken from the side of each stem. The cut surfaces were then quickly bound together with raffia and waxed with a mixture of paraffin and petroleum-jelly. Four weeks later the plants had joined sufficiently so that the raffia and paraffin mixture could be removed without any danger of disturbing the union. In a group of four pairs of plants so treated, successful transfers were obtained in two of the cases. Infection first appeared on that side of the healthy plant where the union occurred. The first symptom, appearing eight days after the stems were bound together, was a yellowing of the veins in the basal portions of those leaves which at the beginning of the experiment were about one-half matured. All subsequent leaves which developed were mottled and showed all of the symptoms typical of the disease on this lily. Leaves which were full-grown at the time of inarching remained normal in all respects. Despite the fact that a union occurred, the other two plants in this experiment failed to give any evidence of infection. The experiment was repeated again with four sets of plants. As in the case of the first experiment, two of the healthy plants became mottled and the other two failed to show infection. As far as is known, this is the first time that mosaic in lilies has been experimentally transmitted.

¹ The work here reported was conducted in connection with the Lily Disease Investigation Fellowship established through the cooperation of the Horticultural Society of New York, The Boyce Thompson Institute for Plant Research, The Department of Plant Pathology at Cornell University, and the New York Botanical Garden.

Following these experiments, attempts were made to effect transmission by means of the juice from diseased plants. It was found that the disease is not difficult to transmit mechanically but that it is apparently not so highly infectious as mosaic of tobacco or cucumber. Well-mottled leaves from mosaic plants were removed and ground in a sterile mortar together with sterile distilled water. Two leaves on each of six healthy plants were then inoculated by pricking and scratching with black enamel insect pins which had been dipped in the juice from diseased plants. After eight days, three of the six plants so inoculated became diseased. All of the six checks remained healthy while the plants were under observation. As in the case of the inarched plants the first symptoms appeared in the form of yellowing of the veins in those leaves which were about one-half matured at the time of inoculation. With the same number of plants, this experiment was repeated, and this time successful transfers were obtained with four of the six inoculated plants, all checks remaining healthy. Similar results were obtained in experiments in which about 300 plants were used of this and several other species of the genus *Lilium*. These will be reported upon later.—CARL E. F. GUTERMAN, Boyce Thompson Institute, Yonkers, N. Y.

Oxidizing agents in sulphur to increase fungicidal activity.—Having obtained partial control of the Helminthosporium disease of sugar cane called eye-spot, with sulphur-dust applications, we attempted late in the winter months of 1925–26 to increase the fungicidal activity of the sulphur by the addition of oxidizing agents. These attempts were sufficiently successful to warrant experiments on a much larger scale, which gave very successful results in the winter of 1926–27.^{1,2}

The present note is for the comparison of these aforementioned results with interesting results obtained by Bailey and Greaney³ with sulphur plus an oxidizing agent for stem rust of wheat in Manitoba. These investigators report that slightly better control of rust was obtained by using oxidized sulphur than by using unoxidized colloidal sulphur. In their experiments two plots received each treatment, and the percentages of rust infection apparently were determined by a visual comparison of the plots by persons with no knowledge of the treatments that had been given so that they could not show prejudice for or against any treatment.

¹ LEE, H. ATHERTON and J. P. MARTIN. The development of more effective dust fungicides by adding oxidizing agents to sulphur. *Science*, 66: 178. 1927.

² ———, and ———. More effective dust fungicides by the use of oxidizing agents with sulphur. *Industr. and Engineering Chem.*, 20: 23. 1928.

³ BAILEY, D. L. and F. J. GREANEY. Dusting with sulphur for the control of leaf and stem rust of wheat in Manitoba. *Sci. Agr.*, 8: 409. 1928.

In this connection we wish to establish in an abstract way a point as follows: In our experiments on sugar cane the degree of infection under the various fungicidal treatments was usually determined by counting the number of infections on from 20 to 40 leaves in a plot, in series of plots with from 6 to 8 or usually 10 replications of the plots for each treatment. These infection counts were then averaged for each leaf, giving us a very good measure of the degree of infection under each fungicidal treatment. The broad leaves of sugar cane, with new leaves forming with fairly uniform regularity, were notably advantageous for such a quantitative determination of the degree of infection.

In this work it was found that visual observation methods of determining the degree of infection were far from being as exact or informative as the quantitative measure of infection obtained by infection counts. We and our associates arrived at the conclusion that we could not observe a difference in the degree of infection in the plots unless the difference exceeded 33 per cent of the amounts of infection. In other words, when treatments gave differences of infection which were readily observable by mere general observation, the differences in degree of infection by actual count usually exceeded 33 per cent.

We therefore desire to call attention to the favorable results which oxidized sulphur gave in the control of eye-spot disease of sugar cane, and to the favorable results which Bailey and Greaney later obtained with stem rust of wheat. We feel that oxidized sulphur will be sufficiently more efficient than the unoxidized sulphur to put dusting operations in more even competition with liquid sprays for prevention of other crop diseases, with the added advantage which dusting operations have over liquid sprays in economy, mobility, and quickness of application. We feel that the control of some of the diseases of other crops could be made more effective and economical by the use of these oxidized sulphur mixtures and wish to ask that other investigators give them a trial.—H. ATHERTON LEE and J. P. MARTIN, Experiment Station of the Hawaiian Sugar Planters' Association, Honolulu, T. H.

*Forced Ventilation as a Means of Controlling Tomato Cladosporium and Septoria in Hotbeds.*¹—Tomato plants grown for 45 days in a manure-heated hotbed and inoculated at two different times² with *Cladosporium fulvum* and *Septoria lycopersici*, but kept dry both day and night by means of an electric heater and electric fan did not become infected with either

¹ Approved for publication by the Director of the Purdue Agricultural Experiment Station.

² Inoculum furnished by Dr. M. W. Gardner, who also confirmed the disease identification.

fungus. Plants grown in an adjacent control bed and similarly inoculated were badly infected with both fungi. Inoculated plants in a third adjacent hotbed were kept free from both diseases by applications of copper lime dust (Niagara D. 25) at weekly intervals.

The use of forced ventilation for controlling leaf spot diseases is of great interest to plant growers and vegetable and flower forcers. It provides an added control measure which helps to insure the control of these diseases under forcing structures.

The circulation of heated air was obtained by drawing air over a 250-watt electric heater by means of a 12-inch electric fan. The heated air with its higher moisture-holding capacity provided a medium for the removal of water from the leaves, including guttation water, even though the relative humidity of the outside air was near the saturation point. The fan, although entirely too large, brought this drier air in contact with all the foliage. On windy and extremely cold nights the heater was effective without the use of the fan. The heater thus served a dual purpose on nights when added heat was most needed. The arrangement of the heater and fan is shown in figure 1.

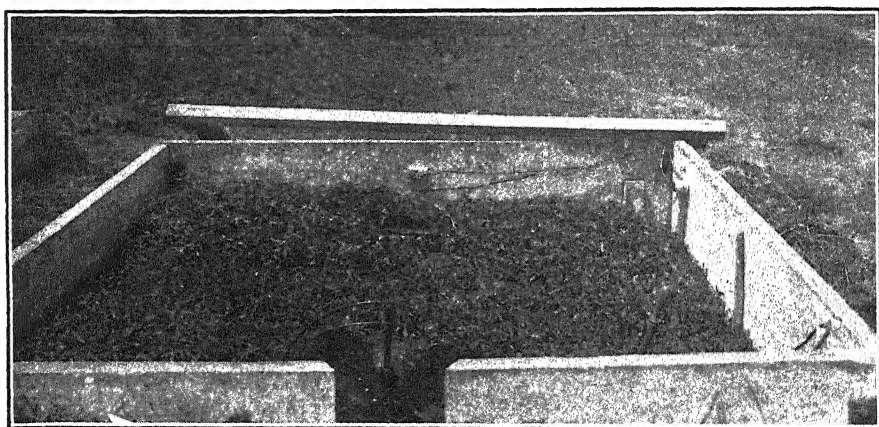


FIG. 1.—Location of electric heater at far end of bed and electric fan used for heating and circulating air in manure-heated hotbed.

Because cold air was being introduced constantly from the outside, the day temperatures in the bed supplied with forced ventilation were 8–10° F. lower than those of the control bed and 2–3° F. lower than those in the bed in which the plants were dusted. The average daily temperature at 11 a. m. was 73° F. in the control bed, in the dusted bed 66° F., and 64° F. in the bed receiving the forced ventilation. This lower temperature is

largely responsible for the production of stockier plants in the bed provided with forced ventilation.

The temperature difference may also be responsible for some of the difference in disease prevalence, although it is our opinion that freedom from disease in the forced ventilated bed is due largely to the lack of moisture on the foliage of the plants. At any rate the conditions existing in the control bed closely approximate those found in many commercial beds.

Although these results are not sufficient to warrant wholesale installation of electric heaters and electric fans in our forcing structures, it does seem that precautions should be taken to provide more ventilation for the purpose of keeping the plant foliage dry and perhaps to keep the temperatures lower. Similar results were obtained by Newhall³ under greenhouse conditions. Replacing the sash during rainy periods for the purpose of keeping plant foliage dry is also suggested. The use of fungicides in addition to the forced ventilation should of course be continued in order to make the control as nearly complete as possible.—E. C. STAIR, H. D. BROWN, T. E. HENTON, Purdue Agricultural Experiment Station.

Permanent mounts of white pine blister rust aecia.—The delicate aecia of *Cronartium ribicola* on pine branches fade and disintegrate in a short time to such an extent that they are soon useless for demonstration purposes. The following method enables one to preserve them in a state closely approximating their normal condition. When the blisters are mature and about to burst, they are treated with a staining solution consisting of about equal parts of orange G and eosin in 95 per cent alcohol. A bit of absorbent cotton twisted around a match or a camel's hair brush may be used to apply the stain. Each blister should be thoroughly impregnated. When the alcohol has evaporated, after about an hour, the same impregnation process is repeated with a 10 per cent solution of paraffin wax in xylol or other similar solvent. Upon drying out, the blisters are surprisingly firm and have a permanent color very like the natural tint.—W. A. McCUBBIN, Pennsylvania Bureau of Plant Industry, Harrisburg, Pa.

Eriksson prizes.—The Committee beg to announce that two prizes are hereby offered for the two best memoirs, giving an account of new and original work on the two following subjects respectively: (1) Investigations on rust (Uredineae) diseases of cereals (wheat, oats, barley or rye); (2) Investigations on the rôle played by insects or other invertebrates in the transmission or initiation of virus disease in plants.

³ Newhall, A. G., Fan ventilation and greenhouse tomato leaf mold. American Produce Grower 3: 5. 1928.

The value of each prize will be 1,000 Swedish crowns. Competitors may be of any nationality. Three typewritten copies of each memoir must be submitted, written in English, French, or German. Memoirs must reach the Secretary of the Committee, Mr. T. A. C. Schoevers, Wageningen, Holland, on or before May 1, 1930.

The author's name must not appear on the memoir itself, but each memoir must be marked with a pseudonym or a motto and the full name and address of the author must accompany the memoir, being enclosed in a sealed envelope bearing on its outside the same pseudonym or motto as is given on the memoir.

The adjudication of the rust prize will rest with a jury, consisting of Professor Dr. Jacob Eriksson, Professor Dr. E. C. Stakman, and Professor M. Et. Foëx. The jury for the virus prize will be announced as soon as possible. The decisions of these juries will be final, and will be announced at the Fifth International Botanical Conference, to be held in Cambridge (England), August 16-30, 1930. The copyright of the prize memoirs will become the property of the committee, who will endeavor to secure publication of them in a suitable periodical or in some other way. Other memoirs will be returned to authors.

The committee reserve the right to withhold the prizes should none of the memoirs submitted be deemed of sufficient merit by the respective juries. Further particulars, if required, may be obtained on application to the Secretary, at the above-mentioned address.

Members of the International Committee for Phytopathology and Economic Entomology: O. Appel, Berlin-Dahlem; J. Eriksson, Stockholm; J. C. F. Fryer, Harpenden; L. Garbowski, Bydgoszcz; E. Gram, Lyngby; L. O. Howard, Washington, D. C.; J. Jablonowski, Budapest; E. de Jaczewski, Leningrad; S. Kusano, Tokyo; L. Mangin, Paris; E. Marchal, Gembloux; P. Marchal, Paris; C. Moreira, Rio de Janeiro; G. H. Pethybridge, Harpenden; L. Petri, Rome; H. M. Quanjer, Wageningen; T. A. C. Schoevers, Wageningen; C. L. Shear, Washington, D. C.—H. M. QUANJER, President.

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